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WATER QUALITY STUDIES

Environmental Protection Agency

May 1974

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WATER QUALITY STUDIES

This course is designed for engineers, chemists, biologists, bacteriologists, and other administrative personnel responsible for the planning and conduct of water pollution surveys.

ENVIRONMENTAL PROTECTION AGENCY
Water Program Operations
TRAINING PROGRAM

May 1974

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TRAINING PROGRAM

Through the Water Programs Operations Office, Environmental Protection Agency conducts programs of research, technical assistance, enforcement, and technical training for water pollution control.

The objectives of the Training Program are to provide specialized training in the field of water pollution control which will lead to rapid application of new research findings through updating of skills of technical and professional personnel, and to train new employees recruited from other professional or technical areas in the special skills required. Increasing attention is being given to development of special courses providing an overview of the nature, causes, prevention, and control of water pollution.

Scientists, engineers, and recognized authorities from other Agency programs, from other government agencies, universities, and industry supplement the training staff by serving as guest lecturers. Most training is conducted in the form of short-term courses of one or two weeks' duration. Subject matter includes selected practical features of plant operation and design, and water quality evaluation in field and laboratory. Specialized aspects and recent developments of sanitary engineering, chemistry, aquatic biology, microbiology, and field and laboratory techniques not generally available elsewhere, are included.

The primary role and the responsibility of the states in the training of wastewater treatment plant operators are recognized. Technical support of operator-training programs of the states is available through technical consultations in the planning and development of operator-training courses. Guest appearances of instructors from the Environmental Protection Agency, and the loan of instructional materials such as lesson plans and visual training aids, may be available through special arrangement. These training aids, including reference training manuals, may be reproduced freely by the states for their own training programs. Special categories of training for personnel engaged in treatment plant operations may be developed and made available to the states for their own further production and presentation.

A bulletin of courses is prepared and distributed periodically by the National Training Center. The bulletin includes descriptions of courses, schedules, application blanks, and other appropriate information. Organizations and interested individuals not on the mailing list should request a copy from The National Training Center.

Introductory Comments about the Training Manual

WATER QUALITY STUDIES

FOR WHOM INTENDED

The manual accompanies courses offered for engineers, chemists, biologists, bacteriologists, and others having responsibilities for planning and working in water pollution surveys. The scope, content, and level are intended primarily for the worker who is relatively new in water quality surveys, and for the more experienced worker who has such specialized responsibilities that he has little opportunity to gain a perspective with regard to the contributions made by various technical specialists in water quality studies.

MODE OF INSTRUCTION

Course participants attend lectures, demonstrations, and panel discussions. Field trips to one or more local sites are included, where students participate actively in selected field survey procedures. In the laboratory, through demonstrations and limited active participation, they gain insight into the nature of many of the phases of required laboratory operations and their interrelationships with field procedures.

SUBJECT MATTER

Major subject areas include:

Identification of pollution problems in the aquatic environment in terms of sources of pollution, and special pollution problems in the geographic area in which the course is presented;

Elements of water quality surveys, including planning, laboratory, field, and reporting aspects. Special emphasis is given to stream surveys, with limited attention to significant features of long-term surveillance studies and special aquatic situations such as lakes and impoundments, coastal waters and tidal estuaries, and ground waters.

Technical subject areas include:

Oxygen relationships, DO, BOD, COD, natural self-purification;

Chemical procedures and evaluations in pollution studies;

Aquatic organisms: significance, applications, methods of measurements;

Bacterial pollution indicators: significance, applications, methods of measurements;

Related physical tests and measurements.

The worker who desires more detailed information on any of the specialized technical subject matter in this course should consider enrollment in a training course limited to the area of his special interest. Several such courses are offered by this program.

National Training Center
Water Program Operations
Environmental Protection Agency

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POLLUTIONAL CHARACTERISTICS OF DOMESTIC WASTE WATERS

I INTRODUCTION

Historically, water pollution activities centered around wastewaters originating from private and municipal sources. Today, our water pollution control activities recognize many sources, including wastewaters originating from industrial operations.

For the purpose of this discussion, it is appropriate that we define the nomenclature used to classify various wastewaters which are discharged to our streams. Many agencies, associations, etc., have postulated definitions of various wastewaters. It is not the intention of this lesson to determine the merits of the various definitions; however, we should recognize that discrepancies do exist. For our purposes, wastewaters may be classified as:

A Sanitary Wastewaters - wastes which originate in the home; this includes bathroom, kitchen and laundry wastewaters.

B Domestic Wastewaters - wastewaters originating from a residential community with the normal amount of commercial and service institutions (banks, shopping centers, coin laundries, service stations, hospitals, etc.). Here the major portion of the wastewaters can be classified as principally sanitary in nature.

C Municipal Wastewaters - wastewaters from municipal complexes where a fair portion (approximately 20% or more) of the wastewaters are from industrial operations with the major portion being domestic sources.

D Industrial Wastewaters - wastewaters from industrial operations where only a slight percent of the wastewaters is from sanitary facilities at the plant.

F Combined Wastewaters - a combination of storm water runoff and sanitary, domestic, municipal, or industrial wastewaters. Surface runoff may often constitute the major portion of such wastewaters.

The terms "wastewater" and "sewage" often are used interchangeably. "Wastewater" reflects the changes in municipal discharges which once were predominately household and commercial, but have changed to include a larger fraction of industrial discharge. Further, the word "sewage" is associated with a certain "aroma" that prompted selection of a different term with a more "cleaned-up" connotation for professional and public use. "Wastewater" is the term accepted and promoted by the Water Pollution Control Federation. Its acceptance is increasing with the term "sewage" becoming less frequently used.

II COMPOSITION OF DOMESTIC WASTEWATERS

A The composition of various wastewaters are of interest to all parties engaged in water pollution control and water resources development. The composition or characteristics of any wastewater determine its hazard to receiving waters and the subsequent value of these waters. The engineer must have information on the characteristics of a waste in order to design and operate treatment facilities. The industrialist should know the characteristics of the wastewater from his plant so he may better evaluate the effects of his plants' wastes upon his neighbors and downstream water users. In the forseeable future, an industry's waste characteristics may be one of the major yardsticks used to ascertain whether or not that industry will be allowed to locate in certain sections of the country. Therefore, the importance of defining or characterizing any wastewater becomes of significant concern.

B Fortunately, domestic wastewaters are fairly uniform in composition and vary only slightly throughout the country.

C Factors that affect the characteristics of domestic wastewaters:

1 Community characteristics

a Amount of available carriage water

In areas where water use per capita is low or where there is a high consumptive use of water for domestic purposes (lawn sprinkling, etc.) slightly higher strength wastewaters may be expected.

b Economic levels

In general, higher cost residential areas contribute more volume of wastewater due to higher usage, except possibly in arid areas where high use may reflect consumptive uses such as lawn sprinkling, etc. Garbage grinders may add significantly to the strength of the wastewater.

2 Collection system

a Separate sewer system - sanitary wastewaters and storm run-off carried in separate sewers.

b Combined sewer system - one sewer used to carry both sanitary wastewater and storm run-off.

c Condition of system - age, grades, infiltration and exfiltration rates, etc.

d Detention time in collection system - detention time or time of travel from the point of collection to the point of discharge or treatment may significantly influence the characteristics of the wastewater. Long times of travel may result in septicity and result in odor problems.

3 Daily and hourly fluctuations in rate of flow will naturally affect the concentrations that may be expected.

D Characteristics of Domestic Wastewater

1 Table I illustrates the constituents of major concern normally found in domestic wastewaters and gives some idea of the range of concentrations that might be expected.

2 Wastewaters are usually characterized under three major classification schemes:

a Physical characteristics

b Chemical characteristics

c Biological characteristics

3 Domestic wastewater characteristics- Note - all values in mg/l except as noted.

III DOMESTIC WASTEWATER CHARACTERISTICS AFTER CONVENTIONAL TREATMENT

Four components will be discussed because in most cases they are of prime concern from a pollution viewpoint. The three components of main interest are bacteria (coliform), nutrients (including biochemical oxygen demand, nitrogen, phosphorus) and solids.

A Bacterial concentrations - coliforms are indicator organisms used to predict the possible presence of pathogenic (disease) organisms. They are important from a public health standpoint. It should be noted that coliforms are only a portion of the organisms present.

B Nutrients

1 Biochemical oxygen demand (BOD) - by definition a measure of the amount of oxygen required by organisms to

Table 1. CHARACTERISTICS OF DOMESTIC WASTEWATERS

Constituents	Weak	Medium	Strong
Physical characteristics			
Color (non-septic)	Gray	Gray	Gray
Color (septic)	Gray-black	Blackish	Blackish
Odor (non-septic)	Musty	Musty	Musty
Odor (septic)	Musty - H_2S	H_2S	H_2S
Temperature - °F **	55° - 90°	55° - 90°	55° - 90°
Total solids	450	800	1200
Total volatile solids	250	425	800
Suspended solids	100	200	375
Volatile suspended solids	75	150	200
Settleable solids	2	5	7
Chemical characteristics			
pH - (units)	6.5	7.5	8.0
Cl, SO_4, Ca, Mg, etc.*			
Total nitrogen	15	40	60
Organic nitrogen	10	25	40
Ammonia nitrogen	-	0.5	1.0
Nitrate nitrogen	-	0.5	1.0
Total phosphate - PO_4	5	15	30
Biological characteristics			
Total bacteria $(\frac{\text{counts}}{100\text{ ml}})$	1×10^8	50×10^8	100×10^8
Total coliform $(\frac{\text{MPN}}{100\text{ ml}})$	1×10^6	50×10^6	100×10^6
Biochemical oxygen demand (5-day)	100	200	450

* Quite variable depending on natural water quality of region.

**For the Central States zone.

stabilize degradable organic material. BOD is an indirect measure of the amount of degradable organic matter present and a fairly good technique for measuring the oxygen demand of a waste. Various modifications of the BOD test are popular.

2 Nitrogen is a normal component of sanitary wastewater. Discharge of nitrogen in the form of ammonia may increase the burden upon the oxygen resources of streams. Nitrates may foster the growth of undesirable aquatic plants.

3 Phosphorus is naturally present in sanitary wastewaters and with the advent of synthetic detergents where phosphates are an important ingredient higher phosphorus concentrations are now found in domestic wastewater than in previous years.

C Suspended Solids - These are important from an aesthetic viewpoint (floating solids, sludge banks, etc.) and represent a source of organic matter (BOD) in receiving waters.

B Wastewaters from Primary Treatment Plants*

Constituent	Expected range of removal
Coliforms	50 - 60%
Solids - total suspended	35 - 65%
BOD	25 - 40%
Nitrogen	
Organic - N	20 - 40%
NH_3 - N	-
NO_3 - N	-
Total phosphate	-

*No chlorination.

C Wastewater from Secondary Treatment Plants*

Constituent	Expected range of removal
Coliforms	70 - 95%
Solids - total suspended	70 - 90%
BOD	65 - 95%
Nitrogen **	
Organic - N	65 - 80%
NH_3 - N	50 - 90%
NO_3 - N	+ 200 - 500 % increase
Total phosphate **	20 - 60%

* No chlorination.

** Removal primarily associated with fresh solids removal and control of process feedback.

IV CHARACTERISTICS OF STORM WATER RUNOFF

Increasing emphasis is being given to the problem of storm water contamination due to wash off of accumulated surface refuse. This cannot be neglected in the control of surface water quality. (see tables below).

Constituent Concentrations in Urban Land Runoff at a Cincinnati Sampling Point, July 1962 through Sept. 1963*

Parameter		Range	Mean
		(units)	
Turbidity		30-1,000	170
Color		10-380	81
pH		5.3-8.7	7.5
		(mg/l)	
Alkalinty		10-210	59
Hardness (as $CaCO_3$)	Ca	24-200	63
	Mg	2-46	15
	Total	29-240	78
Cl^-		3-35	12
SS		5-1,200	210
VSS		1-290	53
COD		20-610	99
BOD		2-84	19
Nitrogen (as N)	NO_2	0.02-0.2	0.05
	NO_3	0.1-1.5	0.4
	NH_3	0.1-1.9	0.6
	Org.	0.2-4.8	1.7
PO_4 (total soluble as PO_4)		0.07-4.3	0.8

* January and February not included.

Bacterial Counts in Stormwater Runoff Samples from an Urban Area*

	Counts Exceeded in Designated Percent of Samples (Colonies per 100 ml)		
	90%	50%	10%
Coliforms†	2,900	58,000	460,000
Fecal coli†	500	10,900	76,000
Fecal strep‡	4,900	20,500	110,000

* Samples were taken from the Cincinnati study site during 1962 and 1963.

† Coliforms and fecal coliforms were determined by the completed MPN technique during 1962, and by the MF technique during 1963

‡ Fecal streptococci were detected by KF streptococcus agar pour plates in 1962 and 1963.

Comparison of Stormwater Runoff Loads and Sanitary Sewage Loads (lb/yr/acre)*

Constituent	Stormwater Runoff†	Sanitary Sewage (raw)‡	Storm % Sanitary
SS	730	540	140
VSS	160	360	44
COD	240	960	25
BOD	33	540	6
PO_4	2.5	27	9
Total nitrogen —N	8.9	81	11

* Multiply by 1.12 to obtain kg/yr/hectare.

† Stormwater runoff—Based on essentially complete measurement of rainfall and consequent runoff water quantity and quality at the study site during September through November 1962, and March through September 1963, projected to average annual rainfall at Cincinnati.

‡ Sanitary sewage—Based on population density of 9 persons/acre (22/hectare) and flow of 100 gpd/cap (0.4 cu m/day/cap).

Assumed raw sewage strengths: SS—200 mg/l; VSS—130 mg/l; COD—350 mg/l; BOD—200 mg/l; PO_4—10 mg/l; Total N—30 mg/l.

V SUMMARY

Pollution caused by wastewaters from urban centers are still the major concern in the United States since natural water quality is most severely stressed at these locations. Nutrient enrichment, plant and animal growth tend to exceed natural stablization capacity of the surface water. Partially treated or untreated wastewaters are primary considerations. Secondary treated discharges frequently are too rich in nutrients such as nitrogen or phosphorus for discharge without additional treatment. Storm water runoff requires major consideration in the wastewater renovation program.

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This outline was prepared by Peter F. Atkins, Jr., Sanitary Engineer, formerly with the National Training Center, and reviewed by F.P. Nixon, formerly Acting Regional Training Officer, Northeast Regional Training Center, EPA, WPO, Edison Water Quality Research Laboratory, Edison, NJ 08817.

Descriptors: Domestic Wastes, Liquid Wastes, Municipal Wastes, Pollutants, Sewage Effluents, Sewage Treatment, Storm Runoff, Storm Water, Surface Runoff, Urban Runoff, Wastes, Waste Treatment, Waste Water(Pollution), Waste Water Treatment, Water Pollution, Water Pollution Sources

SOURCES AND EFFECTS OF INDUSTRIAL WASTES

I INTRODUCTION

As a result of manufacturing every industry produces some kinds and quantities of liquid-borne wastes. These may be detrimental to quality of the receiving waters in many ways: toxicity, oxygen demands, sludge deposits, surface films, thermal effects, etc. Since agriculture is a form of production, the effects of drainage from the land will be considered among the industrial wastes.

II CLASSIFICATION SCHEMES

Classification is one of the most important steps in any water pollution control investigation involving industrial waste discharges.

A There are essentially two approaches to industrial waste classification:

1 Classification according to industry.

2 Classification according to the effects the waste produces in the receiving stream.

B By Industry

Under this scheme industrial wastes are classified as those from the paper and pulp industry, or antibiotic industry, or citrus concentrate industry, etc.

1 As a whole, the manufacture of a specific product will result in similar liquid waste materials. Classifying wastewater by specific industries is very valuable in that it allows for an evaluation of the pollutional load being discharged to a stream no matter where the industry is located. In this regard, calculated waste loadings should be related to plant production, e. g., pounds of BOD per ton of finished product.

2 This classification scheme is based on the assumption that although manufacturing plants within an industry will produce different quantities of waste, the chemical constituents of the wastes will be fairly uniform. Wastewaters from various plants within the same industry will have different effects on water quality when discharged to specific, unique receiving streams.

3 This classification gives no indication of interaction type effects produced by more than one industry within a specified area.

C By Effect

There are many classification schemes for industrial wastes based upon their effects in the receiving water body. This type of classification has more advantages for water quality studies than the previous type, i. e., by industry. Many of the analytical procedures record the effects of the industrial waste (BOD, Bioassay, etc.), rather than the components.

III CLASSIFICATION BY EFFECTS

Classification by effects is based on the supposition that an industrial waste may be characterized by a single predominant effect. In some cases, this may be true, but most industrial wastes exhibit multiple effects. Classification according to one effect may be unduly restrictive. Notwithstanding this problem, classification by effect appears to be the more popular scheme because it is more related to stream conditions and is easily applied.

A Oxygen Depleting Wastes

1 Effect: Low DO levels, due to oxygen demand, may alter the whole balance of the biota of the receiving stream. The natural process of self-purification may be delayed significantly because of the lack of adequate oxygen resources. The demand exerted may be either chemical or biochemical.

2 Typical Wastes: Examples of industrial wastes having significant oxygen demand are:

a Sulfite waste liquors from pulp mills

b Canning plant wash effluents

c Meat packing wastes

d Textile scouring and dyeing effluents

e Milk products wastes

f Fermentation wastes

3 Measurement: Since the primary effect of these materials is a reduction in dissolved oxygen, the BOD test is commonly used to determine the deoxygenation potential of the waste. In conjunction with the BOD, the COD test will yield valuable information.

4 Treatment: All of the methods for the treatment of oxygen depleting wastes involve satisfying all or part of the oxygen demand before discharge to the stream. This usually involves some type of secondary treatment either chemical or biological.

B Toxic Wastes

1 Effects: For the purpose of classification, toxicity is considered as a direct lethal effect on biological forms, as contrasted to the indirect effects of oxygen deficiency or smothering.

2 Typical Wastes: Among the many wastes having toxic effects on stream biota are:

a Spent plating solutions containing heavy metals and cyanides.

b Acid wastes from pickling operations, chemical manufacture, and mine drainage.

c Organic materials such as strong phenolics, antibiotics, pharmaceuticals and petro-chemical wastes, and pesticides.

d Textile dyes

3 Measurement: Traditionally, toxic wastes are evaluated by chemical analysis of the waste to determine the concentration of the offending compound. Unfortunately, suitable analytical methods are not available for some of the newer, more complex materials. The bioassay is rapidly assuming a key place in the evaluation of toxic wastes.

4 Treatment: Removal of the toxic agent is essential to adequate treatment. Heavy metals are precipitated from plating solutions, cyanide is oxidized by chlorination, acids are neutralized, and organic compounds are oxidized biochemically by the use of acclimatized flora.

C Wastes Causing Physical Damage

1 Effects: Certain industrial wastes cause damage to the stream by physical actions rather than chemical or biochemical reaction. These materials create an unfavorable environment because of the specific physical properties which they possess.

2 Typical Wastes: Physical damage may be associated with the following:

a Wastes from lumbering and mining operations, which contribute large amounts of silt, sawdust, and other insoluble deposits.

b Power plant discharges, raising the temperature of the stream in the vicinity of the outfall.

c Petroleum refinery wastes, which contribute oils and other immiscible liquids, color, and sludge.

d Dyeing operations, which might possibly contribute large amounts of color to receiving streams.

3 Measurement: Wastes which create insoluble deposits in the stream are evaluated in terms of the settleable and total solids tests. Special techniques are required for other wastes, e. g., thermal, oil, and industrial color.

4 Treatment: As in the case of the other types of wastes, changing the properties which cause damage is the accepted method of treatment. Settling basins and ponds, cooling systems, oil separators, and similar devices satisfactorily remove the objectionable property.

D Wastes Producing Tastes and Odors

1 Effects: Many industrial effluents contain substances which are capable of imparting disagreeable tastes to water supplies. Even very minute amounts of these materials may cause public reaction. Often the undesirable tastes are increased by chlorination.

2 Typical Wastes: The following contribute tastes and odors to water supplies.

a Petroleum and petro-chemical wastes, even when present in extremely small concentrations.

b By-product coke plants discharges (phenolics) which are rendered very objectionable by chlorination.

c Liquid wastes from the manufacture of synthetic rubber are often implicated where offensive odors are present.

3 Measurement: Because of the small amounts of materials involved, special concentrating techniques, such as the carbon filter, are required. Identification of the compounds by means of infrared spectroscopy and gas chromatography are utilized. Threshold odor testing also yields valuable information.

4 Treatment: Most of the compounds which produce tastes and odors are organic and lend themselves to biological treatment. Certain wastes (those from synthetic rubber manufacturer for example) are resistant to attack and require special treatment processes.

E Waste Containing Inorganic Dissolved Solids

1 Many industrial effluents contribute large amounts of cations (Na, K, Ca, Mg, Fe) which may cause some distress to the stream biota.

2 Typical wastes: Tannery wastes and irrigation waters.

3 Measurement: These wastes are usually evaluated by chemical analysis for the specific cation.

4 Treatment: chemical treatment, coagulation and ion exchange. Irrigation waters pose a special problem as far as treatment is concerned and no adequate treatment technique has been developed.

F Radioactive Waste

1 Effects: Contamination of water environment, destruction of aquatic life.

2 Typical Wastes: nuclear reactor cooling water, uranium ore mining and refining.

3 Measurement and Treatment: Special techniques due to the nature of the contaminant. Types of treatment, ion exchange, storage, coagulation and storage.

G Corrosive Wastes

1 Effects: Highly acidic or basic wastes, although also toxic, deserve special attention as corrosive wastes. Such wastes seriously damage piping systems and may even corrode ships' hulls and bridge piers.

2 Typical Wastes: Examples of corrosive wastes are:

a Spent pickling solutions

b Alkali discharges produced during manufacture of soap.

H Pathogenic Wastes

1 These wastes may contain organisms pathogenic to man or plants.

2 Typical Wastes

a Livestock production (cattle, poultry, swine, laboratory animals)

b Tanneries

c Pharmaceutical manufacture

d Food processing

3 Treatment: Disinfection with chlorine or other active agent.

IV CLIMATOLOGICAL CONDITIONS

Many effects of industrial wastes may be diminished or enhanced by climatological conditions. Some of these to be evaluated are:

A Amount and Frequency of Rainfall

B Amount and Pattern of Run-off

C Stages and Pattern of Stream Flow

D Temperature Patterns - Stream and Air

E Sunlight Patterns

F Wind Patterns

V MISCELLANEOUS FEATURES OF RECEIVING STREAM

A Physical features of stream - cross-sections, profiles, turbulence, etc.

B Past pollution history of stream most important in evaluating present effect of any new industrial waste.

VI SUMMARY

Before any industrial waste can be adequately treated, some idea of the wastes characteristics must be known. The most common procedure is to evaluate the wastewater in light of the condition of the receiving water and the uses to which the receiving water will be put by downstream users. Therefore, approaching the problem based upon the detrimental effects the wastewater produces in the stream and eliminating the causitive agent is the most logical method of attack.

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This outline was prepared by P. F. Atkins, Jr., Sanitary Engineer, formerly with EPA Training Activities.

Descriptors: Industrial Wastes, Liquid Wastes, Pollutants, Waste Treatment, Waste Water (Pollution), Waste Water Treatment, Wastes, Water Pollution, Water Pollution Sources

INFLUENCE OF LAND RUNOFF ON WATER QUALITY

I Earthlife as we know it requires large quantities of water of the right quality, in the right place, at the right time (SINGER, 1968).

A Each person has high, average daily demands.

1 We each need to ingest about two quarts of water per day.

2 To sustain the food chain from the soil to the average person's stomach requires 2,500 gallons per day.

3 Adding the amount required to produce his clothing increases the average per capita water demands to 3,000 gallons per day.

4 The production of creature comforts requires other prodigious amounts.

a Refining 1 gallon of oil requires 18 gallons of water.

b Making a barrel of beer requires 300 gallons of water.

c Steam-powered generating plants use 191,700 gallons of water for each ton of coal that it burns.

d Since it takes 60,000 gallons of water to produce a ton of paper, a large paper mill needs as much water (60,000 × 1,000) as a city of 300,000 persons (300,000 × 200).

B The water supply of the Earth totals a staggering 326,000,000 cubic miles of water (NACE, 1967).

1 Oceans, inland seas, glaciers, and polar icecaps account for 99.37 percent of this supply.

2 Ground water, vadose water, and soil moisture make up 0.62 percent.

3 Fresh-water lakes and rivers have an average instantaneous amount equal to 0.01 percent.

4 The average, instantaneous, atmospheric moisture, the source of all precipitation upon the earth, amounts to only 0.001 percent of the earth's supply of moisture.

II Precipitation, extremely variable in amount, but usually of high quality, is the main source of the world's land runoff.

A In the United States, the average annual precipitation is relatively generous but it is unevenly distributed.

1 It averages about 30 inches per year.

2 It varies from virtually zero in the arid interior deserts to more than 150 inches at the tops of some of the high elevation mountain ranges of the Pacific Northwest.

B Cyclonic storms extend across large areas.

1 These "general" storms move from west to east across the United States.

2 These storms usually have fairly uniform characteristics but are subject to marked orographic effects.

C Convection-type storms tend to originate in certain areas and to traverse somewhat similar paths time after time.

1 Convection storms are relatively small, short, and intense and are much more common in some areas than in others.

2 Convection storms have peak intensities for short periods and these peaks decrease as longer duration periods and shorter frequencies (expected return periods) of the storms are used.

3 An estimated rainfall intensity for various duration periods and frequencies may be calculated from local records.

a To do this, the intensities of all storms on record must be listed in descending order, for the duration period in question, and an ascending order number assigned to each storm.

Example 1. Rainfall intensities at Cincinnati, Ohio during different peak periods of 1-year and 50-year frequency (return period) storms:

Duration Period (Minutes)	Intensities (inches per hour)	
	Once per year	Once in 50 years
5	3.94	9.40
10	3.18	8.60
15	2.55	7.42
30	1.65	5.20
60	1.02	2.60
120	0.61	1.51
1440	0.093	0.21

Example 2. Rainfall intensities at Cincinnati, Ohio during 5 and 120-minute periods of storms with different frequencies (return periods):

Frequency (Years)	Intensity (inches per hour)	
	5-min. period	120-min. period
1	3.94	0.61
2	4.51	0.72
5	5.50	0.90
10	6.38	1.14
25	7.38	1.34
50	9.40	1.51

Example 3. Five-minute rainfall rates, for a 52-year record, in descending order and their assigned, ascending order numbers:

Assigned Order Number (N)	Rainfall rate during the most intense 5-minute period of each storm (I--inches per hour)
1	9.72
2	7.32
3	7.32
4	6.60
5	6.48
6	6.31
7	6.23
etc.	etc.

b Choose F, the frequency (return period) desired and calculate N from the equation $F = (M+1)/N$

where F = frequency (return period) in years,

M = years of record, and

N = order number.

Example 4. Determine N for a 10-year frequency and 5-minute intensity for the 52-year record used in Example 3.

$$10 = (52+1)/N; \quad N = 53/10 = 5.3$$

Interpolation from the table shown in Example 3 using an N of 5.3 as calculated in Example 4 gives an intensity of 6.43 inches per hour.

D Contamination of Rainfall is usually relatively low.

Example 5. Constituent concentrations in a 0.35-inch rainstorm that fell July 12, 1964 at Cincinnati, Ohio:

Constituent	Concentration	Constituent	Concentration
pH	4.2	Nitrite Nitrogen $(NO_2 - N)$	0.0 mg/l
Alkalinity	*0.0 mg/l	Nitrate Nitrogen $(NO_3 - N)$	0.4 "
Total Solids	6.0 "	Ammonia Nitrogen $(NH_3 - N)$	0.4 "
Volatile Solids	3.6 "	Organic Nitrogen (Org - N)	0.8 "
Chemical Oxygen Demand (COD)	3.3 "	Total PO_4	0.26 "

* Milligrams per liter

E Rainfall frequency, duration, intensity, and amount are used in various combinations with other physical, chemical, and biological data to provide the basis for a number of engineering design purposes.

III The instantaneous volume of runoff draining from the world's land surface at any one time is a relatively small amount of the world's total water supply.

A Overland flow from an undisturbed rural environment is usually negligible.

1 Heavily vegetated areas cushion raindrop impact and keep the water clean.

2 Heavily vegetated areas have porous soils and are able to absorb clean water rapidly.

B Land use can greatly increase overland flow.

1 A good road surface is impervious to water.

2 Tillage practices cause greatly accelerated overland flow.

a Clean cultivation permits maximum raindrop impact and soil surface sealing.

b Cultivation causes soil compaction and it is also conducive to concrete frost formation.

c Up-and-down slope tillage concentrates overland flow and accelerates runoff.

d Logging, strip-mining, and other land use retards infiltration and augments runoff.

3 Numerous USDA soil and water conservation studies and practices provide information relative to the relation of land use and management to overland flow and subsurface drainage.

4 Preliminary PHS runoff/rainfall ratios for five small agricultural watersheds varied from 0.11 to 0.33 for a 2.21-inch rainstorm and from 0.20 to 0.56 for a 5.09-inch rainstorm.

C Drainage water from land areas differs greatly in quality compared to precipitation.

1 The agricultural watersheds at Coshocton, Ohio have provided some pertinent information and show that certain constituents have special significance.

Example 6. Quality of drainage water resulting from a rainstorm on one of the agricultural watersheds under study by the USDA and PHS at Coshocton, Ohio:

Constituent	Concentration
Turbidity	90 units
Color	260 "
pH	7.7
Alkalinity	100 mg/l
Hardness (as Ca CO_3)	
Calcium	73. "
Magnesium	63. "
Total	136. "
Chloride	28. "
Chemical oxygen demand (COD)	159. "
Biochemical oxygen demand (BOD)	23. "
Total solids	392. "
Volatile solids	193. "
Suspended solids	56. "
Volatile suspended solids	31. "
Nitrite nitrogen (NO_2 - N)	0.1 "
Nitrate nitrogen (NO_3 - N)	4.9 "
Ammonia nitrogen (NH_3 - N)	0.4 "
Organic nitrogen	7.5 "
Total hydrolyzable PO_4	2.7 "
Ortho PO_4	
Total coliform bacteria	19,000. colonies per 100 ml
Fecal coliform bacteria	340,000 " " " "
Fecal streptococci bacteria	1,300 " " " "
Total organic chlorides	248 nanograms per liter
Lindane (possibly)	32 " " "
2, 4-D Ester (possibly)	141 " " "

a The nitrate, phosphate, and fecal coliform concentrations were high.

b The total organic chlorides could be of some concern.

2 Intensive sampling would be required in order to determine total loads of constituents lost in the runoff.

3 Livestock feedlots are troublesome in some areas (SMITH, 1968).

a The Kansas Board of Health ranks large livestock feedlots as that State's major pollution source.

b Some feedlots contain 10,000 animals.

c Ten thousand cattle have the pollution potential of 225,000 persons.

d Wastes from feedlots have caused fish kills and ruined water supplies of downstream communities.

D Urban development creates an artificial environment with widely varying water pollution potentials.

1 Use and treatment of urban lands and waters varies widely.

a There are 11,500 sewered communities in the 50 United States.

b These communities range in size from less than 0.5 to about 454 square miles.

c Their aggregate area equals about 43,100 square miles, or about 1.2 percent of the total area of the United States.

d Runoff coefficients in urban areas may range from 0.05 for lawns, gardens, and parks, to over 0.90 for roofs, streets, and other solidly-paved surfaces.

e "Design storm" tributary areas to storm sewers are made up of composites of these types of land use.

f About 37 percent of a residential, light-commercial type area is in roofs, streets, and parking lots and the remainder is in lawns, gardens, and parks.

g The entire surface area of some commercial urban areas may be covered with impervious materials.

2 About 83 percent of the 11,500 sewered U.S. communities (mostly the smaller communities) have "separate" sewers and the other 17 percent (mostly the large and old communities) have "combined" systems, or a mixture of "separate" and "combined" systems, with the mixture varying from almost all "separate" to almost all "combined".

a "Separate" sewer systems keep the sanitary sewage separated from the storm sewage so they can be handled independently.

b "Separate" systems have appealed to small communities because they could use a smaller conduit for the sanitary sewage if they used roadside ditches for storm water runoff.

c "Combined" systems have a common collector for both the sanitary and storm water sewage.

d During dry weather combined systems divert all the sewage and the intercepted drainage water to the treatment plant.

e During wet weather the volume of mixture delivered for treatment may be several times as large as the dry weather volume.

f During heavy storms an untreated volume of the mixture 100-200 times larger than that treated may overflow to the receiving streams.

g For some cities, "combined" systems are simpler and cheaper and they were often adequate years ago when all or at least part of the flow could be discharged without treatment.

3 Quantity of storm water runoff can be estimated by the "rational" method,

Q = CIA where:
C = runoff coefficient
I = rainfall intensity
A = drainage area

a The runoff coefficient (C) depends on surface conditions.

Example 7. Runoff coefficients for different kinds of urban surfaces.

Kind of Urban Surface	Runoff Coefficient
Mercantile	0.70 - 0.95
Commercial	0.60 - 0.85
Industrial	0.55 - 0.80
Residential (apartments)	0.5 - 0.7
Residential (single and double dwellings)	0.25 - 0.6
Parks	0.25
Undeveloped areas	0.05 - 0.25

b The rainfall intensity (I) is usually expressed as inches per hour and one inch of rainfall per hour is equivalent to a runoff flow of 1.008 cubic feet of water per second per acre.

c A "design" rainfall rate frequently used is that maximum which may be expected in the particular return period that has a duration period equal to the concentration time of the watershed point being considered.

Example 8. Using a 15-minute concentration time, a 0.5 runoff coefficient, and a rainfall rate (for a one-year return period and a 15-minute duration period) of 2.55 inches per hour (equivalent to 2.57 cfs) the flow per acre would be: (0.5) (2.57) (1) = 1.28 cubic feet per second. In comparison, a residential area sewage discharge at a population density of 10 per acre would be approximately (10) (100) (1/7.48) (1/86400) = 0.00155 cubic feet per second. In this case, the storm water runoff amounts to 826 times the sewage flow at this peak runoff rate.

4 Quality of drainage water from a residential, light-commercial area differs greatly in comparison to drainage from an agricultural area. (Compare Example 9 with Example 6).

Example 9. Constituent concentrations in urban land runoff at Cincinnati sampling point, July through December 1962 and March through September 1963 (WEIBEL, ANDERSON, and WOODWARD, 1964)

Constituent	Range		Mean
Turbidity	30-1000.	units	170.
Color	10-380.	"	81.
pH	5.3-8.7		7.5
Alkalinity	10-210	mg/l	59.
Hardness (as $CaCO_3$)			
Calcium	24-200.	"	63.
Magnesium	2-46.	"	15.
Total	26-240.	"	78.
Chloride	3-35.	"	12.
Settleable solids	5-1200.	"	210.
Volatile settleable solids	1-290.	"	53.
Chemical oxygen demand (COD)	20-610.	"	99.
Biochemical oxygen demand (BOD)	2-84	"	19.
Nitrogen (as N)			
Nitrite (NO_2)	0.02-0.2	"	0.05
Nitrate (NO_3)	0.1-1.5	"	0.4
Ammonia (NH_3)	0.1-1.9	"	0.6
Organic	0.2-4.8	"	1.7
PO_4 (Total soluble as PO_4)	0.07-4.3	"	0.8
Bacterial counts	Number of colonies per 100 milliliters exceeded in designated percent of samples.		
	90%	50%	10%
Total Coliforms	2,900.	58,000.	460,000.
Fecal Coliforms	500.	10,900.	76,000.
Fecal Streptococci	4,900.	20,500.	110,000.

Example 10. Comparison of storm water runoff loads * and sanitary sewage loads *, pounds per acre per year: (WEIBEL, ANDERSON, and WOODWARD, 1964).

Constituent	1 Storm Water Runoff	2 Sanitary Sewage (raw)**	Ratio (Col. 1/Col.2)
Settleable solids	730.	540.	1.35
Volatile settleable solids	160.	360.	0.44
Chemical oxygen demand (COD)	240.	960.	0.25
Biochemical oxygen demand (BOD)	33.	540.	0.06
Phosphate (PO_4)	2.5	27.	0.09
Total Nitrogen	8.9	81.	0.11

* Period July 1962 through September 1963, excluding January and February.
** From a residential-light commercial urban area.

a The importance of the BOD load carried by the storm water runoff shown in Example 10 is probably much greater than that shown by the 0.06 ratio of storm water load to raw sanitary sewage load.

b Sewage treatment would likely remove 90 percent of the raw sanitary sewage BOD.

c The 33 pounds per acre per year load of BOD in the storm water runoff would then be equivalent to 0.6 of the 54 pounds per acre per year of BOD load in the treated sewage effluent.

d The storm water runoff shown in Example 10 is from a relatively "clean" type of land use. Densely populated and industrial urban areas would probably produce more heavily polluted storm water runoffs.

e Studies of stream water quality indicate that coliform bacteria counts increase as turbidity increases. (GELDREICH, 1966) (KUNKLE and MEIMAN, 1968)

f Turbidity varies almost directly with streamflow. Therefore, much of the bacterial load probably arises from surface wash.

g Drainage from a non-urban area such as the Lake Mendota watershed can furnish sufficient nutrients to fertilize the water to the nuisance aquatic growth-producing level. (MACKENTHUN, 1965 p. 11)

h Some rural water supplies may contain 500 milligrams of nitrate nitrogen per liter. (MACKENTHUN, 1965 p. 11)

i On an agricultural watershed near Coshocton, Ohio, two storms with 2.21 to 5.09 inches of rainfall per storm produced 6,600 to 76,300 gallons of runoff per acre. (MACKENTHUN, 1965 p. 12)

j Phosphate (PO_4) in this runoff water ranged from 0.05 to 0.42 pounds per acre.

k Total nitrogen (N) ranged from 0.20 to 6.12 pounds per acre.

l The use of pesticides has increased agricultural production and improved mankind's health and comfort. (BOGAN, OKEY, and VARGAS, 1961) (HOFFMAN, 1959)

m Pesticides will have to be used with increasing care in order to avoid tragic, secondary effects, however. (WEBB, 1960) (WORRELL, 1960) (GILETT, 1970)

n Pesticides already occupy a prominent position among the list of fish-killing water pollutants. (MCKEE and WOLF, 1963) (PRESSMAN, 1963) (PUBLIC HEALTH SERVICE, 1961) (COTAM, 1960) (BARRY, 1961)

o The compounds DDD, DDE, DDT, chlordane, and endrin were found regularly in samples of both water and biota taken during the pesticide monitoring of the aquatic biota of the Tule Lake National Wildlife Refuge and the occurrence of endrin was directly associated with contaminated irrigation return water supplying the Refuge Lakes. (GODSIL and JOHNSON, 1968)

p Many studies show that some herbicide will appear in nearly all streams which flow by or through treated areas. (NORRIS, 1967)

q A water treatment of soil-applied picloram pellets for the control of chaparral brush resulted in the movement of detectable amounts of picloram into the stream water. (DAVIS, INGEBO, and PASE, 1968)

r The herbicide amitrole, aerially sprayed on 100 acres of a headwater sub-basin of a steep, clearcut, forest watershed in a silvicultural attempt to control salmonberry, was found in measurable amounts in samples of water near the downstream edge of the sprayed area during and for 5 days after spraying. (MARSTON, SCHULTS, SHIROYAMA, and SNYDER, 1967)

5 "Combined" sewer problems may be even more troublesome than those of separate systems.

a Treatment plants are seldom large enough to handle all the volume produced during heavy storms.

b The quantities and flow patterns are comparable to separated storm water flows. However, usually during storm periods, combined systems can handle no more than 1.5 to 5 times the normal dry weather flow.

c When the stormflows exceed this amount (see the 826-fold increase shown in Example 8) some, most, or all of the flow must overflow to the effluent receiving streams.

d During the 1964 Christmas floods many of the sewage treatment plants in the Pacific Northwest were completely inoperative. (MARSTON and NIELSON, 1967)

e Elsewhere it has been claimed that as much as 95 percent of the sanitary sewage overflows during some storms.

f "...about 1/3 of the city's annual production of sewage solids overflowed without treatment although only 2 to 3 percent of the sewage volume actually overflowed..."

g Some "combined" systems have been custom-tailored to provide local relief at certain critical points by diverting part of the load to other points of overflow.

h Most storm sewers and certain parts of most combined systems are of an extremely localized nature.

i The number of overflow points varied from 1 to 42 among 30 cities.

j Little specific qualitative information has been determined for combined sewer overflows.

Example 11. Quality of a combined sewer overflow:

pH	7.1	
Chloride	619.	mg/l
Total solids	400.	"
Volatile solids	144.	"
Settleable solids	203.	"
Sand	76.	"
Dissolved oxygen	6.9	"
Oil and grease	33.	"
Biochemical oxygen demand	59.	"

IV Nearly all of the 17 percent of the United States supply of water which is withdrawn, used and returned to the waterways is polluted to some degree. (SINGER, 1968)

A This restricts its further use and contaminates the receiving waters.

B The streams' self-purification processes are unable to cope with today's massive waste burdens and complex effluents.

C Effective waste management is needed to protect clean waters and to upgrade polluted waters so that they may be reused. The land surface can be used as one method of waste treatment and disposal.

1 The overlying soil and the underlying geological strata may be thought of as a three-dimensional, anisotropic, chromatographic column capable of adsorbing and removing solutes from a percolating solution. (BAILEY, 1968)

a If the interactions between different nitrogen and phosphorus forms in the effluent and soils and geological strata are known, it is possible to predict the chromatographic behavior of the "soil waste treatment system."

b Using such information it would be possible to set forth the criteria for selecting sites of "soil waste treatment systems."

c Similarly it would be possible to indicate how those soils, lacking the necessary critical properties, can be modified to serve as "soil waste treatment systems."

2 An intensive study at Pennsylvania State University has given much factual information on the efficiency of the soil as a filtering medium (PENNYPACKER, SOPPER, and KARDOS, 1967)

a Overland flow causes soil erosion and washes silt and toxicants into the streams.

b Avoid overland flow by increasing infiltration, interflow and sub-flow. (MARSTON and WHIPKEY, 1964). Use sprinkler applications; disperse and control overland flows by the use of grassed waterways.

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Descriptors: Liquid Wastes, Pollutants, Storm Runoff, Storm Water, Surface Runoff, Urban Runoff, Wastes, Water Pollution, Water Pollution Sources

IRRIGATION RETURN FLOW AND AGRICULTURAL RUNOFF

Irrigation is a major use of water in the United States, exceeding that used for domestic and industrial supply. This is largely a situation which exists in western areas since over 90 percent of the irrigated land is located in the arid regions west of the Mississippi River.[3]

When water is utilized for the irrigation of land, a portion of this water is returned to surface or groundwater sources. Water that is applied to the land is known as "irrigation water," and that which returns to the stream or groundwater is known as "irrigation return flow."

Irrigation return flow is a major source of water pollution since it affects the quality of water for subsequent uses such as domestic, industrial and irrigation supply, wildlife and fisheries, and recreation.[2, 4, 7, 9, 11, 12] Our objective in this lecture is to consider the type of water problems which result from irrigation return flow and agricultural runoff and the magnitude and significance of these problems.

I IRRIGATED AREAS OF THE WORLD

The problem of pollution from irrigation becomes significant when we consider the magnitude of the area under irrigation and the quantities of water involved.[3]

A In 1960 it was estimated that there were about 250 million acres under irrigation in the world.

  1 India and China lead the world with approximately 70 million each.

  2 The United States is third with over 36 million acres.

    a Over 32 million acres are in the 17 western states which produce crops valued at over 3 billion dollars annually.

B Irrigated area has increased and continues to increase at a rapid rate.

  1 Irrigated acreage in the United States has increased tenfold from 1890 to 1956.

  2 Overall increase during 2-year period (1954 to 1956) of almost 5-1/2 million acres.

  3 It has been predicted[4] that by 1970, most of the potential area of 51-1/2 million acres in the western states will be under irrigation.

II IRRIGATED AGRICULTURE

A Water used for irrigation may be defined in the following ways:[5, 6]

  1 The gross overall supply required

  2 That which is withdrawn from the source

  3 That which is delivered to the farms

  4 That which is transpired or evaporated from a cropped area (consumptive)

  5 That which returns to surface or groundwater sources (nonconsumptive use)

B Quantity of Irrigation Water Involved

  1 In 1956 the total quantity of water used for irrigation in the 17 western states was estimated to be 128 million acre-feet.[3]

  2 By 1970 it is predicted that this may increase to 170 million acre-feet[3].

  3 In 1960 total water used in the United States was estimated to be 270,000 mgd[6] as follows (two significant figures):

a Rural	3,600 mgd
b Public supplies	21,000 mgd
c Self-supplied industrial	140,000 mgd
d Irrigation	110,000 mgd
1) Conveyance losses	25,000 mgd
2) Delivered to farms	85,000 mgd

C Quantity of Irrigation Return Flow

  1 Irrigation is a consumptive use of water inasmuch as an average of about two-thirds of the water diverted to the farm is lost through evaporation from water and land surfaces, and by transpiration by plants (evapotranspiration).

2 An average of about one-third of the water diverted for irrigation returns to surface streams or groundwater. The actual quantity may vary from 20 to 60 percent of that applied, depending on location, soil characteristics, climatic conditions, and management practices.

3 Based on above figures, this totals about 30,000 mgd of return flow. If this all occurs in a 3- to 4-month growing season, then the daily total is more like 110,000 mgd (about equal to municipal and industrial waste flow for the same period).

4 This indicates quantitatively the magnitude of the irrigation return flow problem.

III IRRIGATION RETURN FLOW

A Sources of Return Flow

1 The flow of water through a typical irrigation project is shown by the flow diagrams involving surface and groundwater sources in Figure 1.

2 Water used consumptively:

- a Evaporation from water and land surfaces
- b Transpiration by plants

3 Three sources contributing to return flow:

- a Overflow or wastage from canals usually returns directly to stream.
- b Runoff is the water applied in excess of the infiltration capacity of the land. It may be high in turbidity due to erosion and may contain fertilizers, organic matter, and other contaminants washed from the land.
- c Seepage or drainage is the water which passes through the soil and may either return to the surface stream or accumulate in the groundwater strata. This portion of the return flow is significantly changed in quality--especially in mineral content.

B Changes in Quality

1 The most significant changes in quality are:

- a Increase in mineral content
- b Increase in temperature, turbidity, color, and taste
- c Increase in nutrients which promote aquatic growth
- d Presence of nitrates in concentration of health significance
- e Potentially toxic quantities of numerous pesticide materials.

2 Whether or not the above changes in quality constitute a pollution problem depends on the specific situation and the use to be made of the receiving water.

C Mineral Salts (Salinity)

1 Return flows contain at least three, and often as high as ten, times the concentration of mineral salts as that of the initial irrigation water.

2 Loss of water through evaporation and transpiration accounts for a major portion of this increase since the salts are contained in a lesser amount of water. This is illustrated in Figure 2.

3 The water of many western streams is used several times for irrigation, thereby increasing the opportunity for further concentration of the salts. The Rio Grande is a classic example of this effect of irrigation on the mineral content of a stream. The data of Table 1 show this effect at 7 sampling points from Otowi Bridge near Santa Fe to Fort Quitman below El Paso.

Table 1

SALINITY OF RIO GRANDE FROM 1945 SURVEY*

Item	Sampling Points						
	1	2	3	4	5	6	7
Discharge (1,000 acre-ft.)	1,132	814	840	883	814	596	208
Dissolved salts (mg/l)	190	380	430	480	510	802	2,000
Dissolved salts (1,000 tons)	294	420	492	578	565	620	582

*U. S. Salinity Laboratory, Riverside, California.[3]

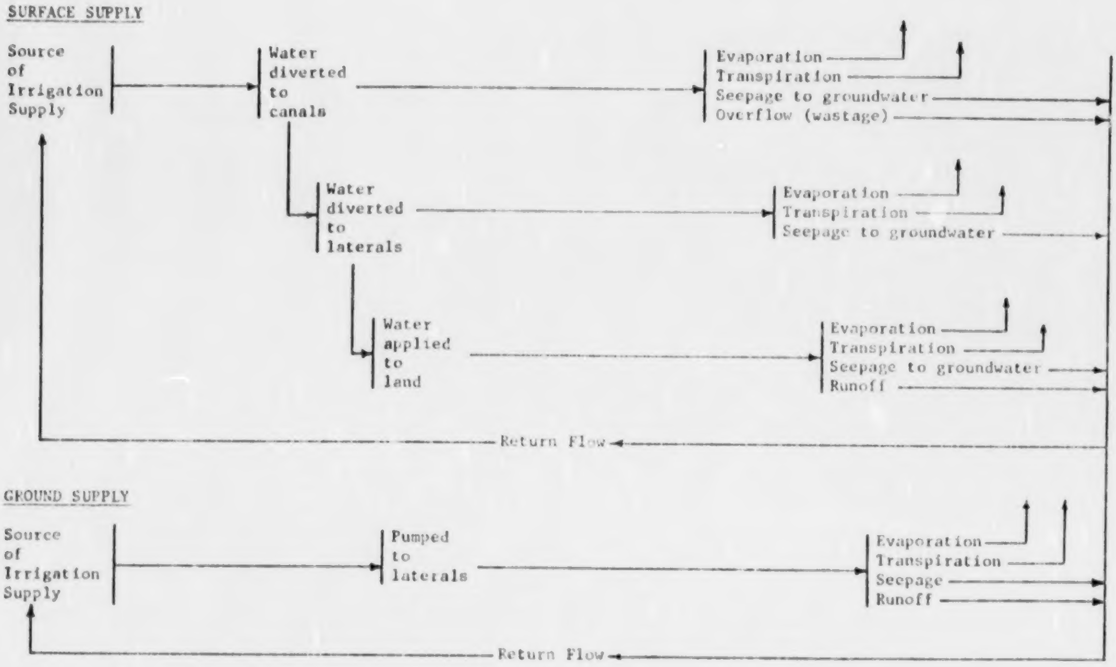
SURFACE SUPPLY
Source of Irrigation Supply
Water diverted to canals
Evaporation
Transpiration
Seepage to groundwater
Overflow (wastage)
Water diverted to laterals
Evaporation
Transpiration
Seepage to groundwater
Water applied to land
Evaporation
Transpiration
Seepage to groundwater
Runoff
Return Flow
GROUND SUPPLY
Source of Irrigation Supply
Pumped to laterals
Evaporation
Transpiration
Seepage
Runoff
Return Flow

Figure 1. FLOW DIAGRAMS FOR TYPICAL IRRIGATION PROJECTS[3]

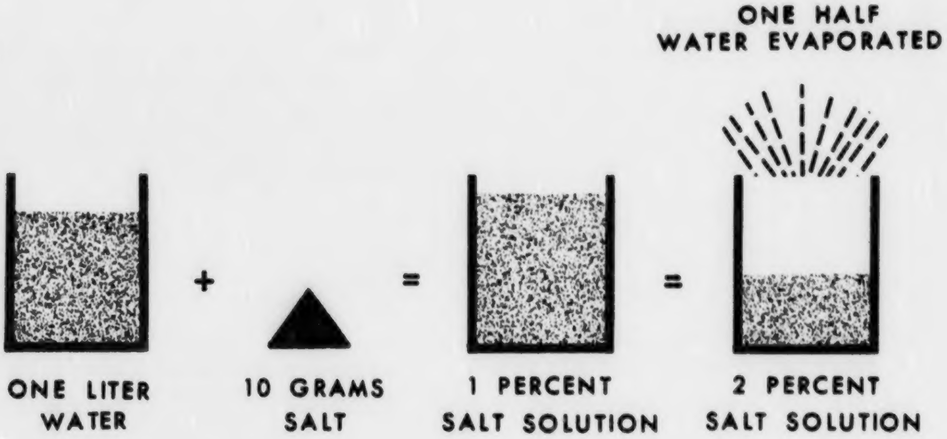
ONE HALF WATER EVAPORATED
ONE LITER WATER
+
10 GRAMS SALT
=
1 PERCENT SALT SOLUTION
=
2 PERCENT SALT SOLUTION

Figure 2. EFFECT OF EVAPORATION (AND/OR TRANSPIRATION) ON THE CONCENTRATION OF SALT SOLUTION.

4 The growth of plants is adversely affected by high salinity, therefore, it is necessary to flush these salts from the soil continually by the application of water over and above the plant requirements. This procedure is known as maintaining a "salt balance"[8, 13, 14] which to be favorable requires that the salt output from the project be equal to or greater than the input. The smaller volume of water returning to the stream must contain all the salts added with the irrigation water plus any which may dissolve from the soil particles.

5 Data for the Yakima River in Washington are shown in Table 2. The percentage increase in salinity is about the same as for the Rio Grande; however, the range of values is lower because of the low concentration of salts in the initial irrigation water.

Table 2

SALINITY, YAKIMA RIVER, 1951*

Sampling Station	Miles Travel	Dissolved Salts (mg/l)
Easton	0	41
Cle Elum	17	49
Ellensburg	44	63
Rosa Dam	71	51
Wapato Dam	92	74
Toppenish	108	326**
Granger	115	348
Sunnyside	128	358

*Data from Washington Pollution Control Commission.

**Major return flows enter above this location.[3]

6 Along with increased salinity, there also occurs an increase in hardness. The effect of irrigation on total and permanent hardness in several different areas is shown in Table 3.

A hardness value above 200 mg/l as $CaCO_3$ is not satisfactory for domestic or most industrial uses.

D Temperature

1 Temperature is a critical characteristic of water from the standpoint of the fishery and, in many cases, of domestic and industrial supply.

Table 3

EFFECT OF IRRIGATION ON TOTAL AND PERMANENT HARDNESS

Location	Total Hardness (mg/l)*		Permanent Hardness (mg/l)*
	Above Irrigation	Below Irrigation	
Rio Grande, Texas	111	631	500
Yakima River, Wash.	33	134	50
Sunnyside Project, Wash.	40	299	188
Arkansas River	212	890	650
Sutter Basin, Calif.	72	480	208

*As $CaCO_3$ [3]

2 Increased water temperature causes a decrease in dissolved oxygen content.

3 Soil surfaces become very hot in summer.

4 Water traveling over hot soil surfaces becomes heated.

5 If tile drains are used, their effluent may have higher temperature than applied water. Greater temperature increases occur, however, in open drains.[11]

E Turbidity, Color, and Taste

1 Increased turbidity due mainly to soil erosion.

2 Color changes due to dissolved organic materials such as plant residue.

3 Tastes and odors may be imparted to water from organic residues such as algae, microorganisms, etc.[9]

F Nutrients

1 Nutrients in surface waters produce excessive growths of algae and other aquatic plants.

2 Algae and aquatic weeds in ditches and streams below irrigation projects indicate that return flows are a significant contributor to the nutrient problem.

3 The three important elements are potassium, nitrogen, and phosphorus.

4 Silvey[9] reported on studies utilizing "tagged" phosphorus where 5 mg/l of the

element was contained in the drainage from an area to which 25 mg/l was applied.

5 A study[11] of the drainage waters in the Yakima Valley showed that all of the major nutrient elements increased downstream due to drainage from irrigated areas.

G Nitrates

1 Nitrates in water supplies are significant in that concentration in excess of 10 mg/l as N (40 mg/l as NO_3) are dangerous and may be fatal if the water is used in the feeding of infants.

2 Ammonia and organic nitrogen compounds are oxidized by soil microorganisms to nitrates.

3 Nitrates are highly soluble and will move with the soil water.

4 A portion is used by plants, but the unused portion may be contained in the drainage water.

5 The data of Table 4 show how nitrates are increased in the Oxnard Plain Area, Ventura, California. The applied water contained only 3 mg/l as NO_3.

Table 4

NITRATE CONTENT OF DRAINAGE WATER*

Location	Nitrate as NO_3 (mg/l)
Drain at Oxnard Road	28.7
Ditch near Port Hueneme	21.0
Ditch at Bridge 520	49.6
Revilon Slough	56.9
Ditch, Hueneme Road	68.1
Ditch west of West Road	64.5
Ditch south of Nauman Road	84.0
Ditch on Pleasant View Road	74.0

*Data from California Water Resources Board for Oxnard Plain Area.[3]

H Pesticides

1 Insecticides and herbicides applied to plants and land may enter streams in toxic quantities.

2 Fish kills have been reported that were caused by persistent and toxic quantities of these substances.

3 Many are known to persist for long periods in the soil.

4 It is reasonable to assume that some of these may be washed from the soil along with other mineral salts.

IV RUNOFF FROM AGRICULTURAL AREAS

A The Infiltration Process[1]

1 Infiltration is defined as the movement of water through the soil surface into the ground.

2 After entering the soil, it is referred to as soil water (stored) or percolating water (moving downward).

3 Infiltration capacity is defined as the maximum rate at which a given soil can absorb precipitation as it falls.

4 If precipitation exceeds the infiltration capacity, water accumulates on the surface and runoff occurs.

5 Measurement of infiltration is difficult. Indirect measurements are usually made (precipitation minus runoff).

6 Many physical factors affect and/or control the infiltration process.

B Water Pollution Resulting From Agricultural Activities[12]

1 Fertilizers and agricultural lime

a Principal elements are: nitrogen, phosphorus, potassium, calcium, chloride, sulfate, and magnesium.

b These reach water courses in the inorganic farm.

2 Animal waste problems are complicated by concentrating large numbers of animals in a small area as in large feedlots.

a Waste disposal presents a problem.

b One instance reported in which 75 cu. yd. of manure was dumped in a river and approximately 1,000 fish were killed over a distance of 3 miles downstream.[12]

3 Pesticides are a source of great concern in water pollution problems.

  a Include insecticides, herbicides, fungicides, growth regulators, etc.

  b Chlorinated compounds including DDT, endrin, dieldrin, toxaphene, and others are most toxic to fish and are most commonly used. These are degraded slowly in the natural environment.

  c Organic phosphorus compounds such as parathion and malathion are considered more toxic to warm-blooded animals.

  d Much more needs to be known in order to control pollution from these sources.

C Nutrient Content of Drainage Waters[10]

1 Sylvester[10] has reported on variations in nutrient content from forested, urban, and agricultural areas.

2 Streams from forested areas are lowest in nutrient content.

  a Lowest in soluble phosphate.

3 Urban street drainage was highest in total Kjeldahl nitrogen.

4 Highest total phosphorus found in surface irrigation drains.

5 Highest soluble phosphate and nitrate found in subsurface irrigation return flow drains.

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Descriptors: Fertilizers, Irrigation, Irrigation Effects, Irrigation Systems, Leaching, Liquid Wastes, Pollutants, Soil-Water-Plant Relationships, Return Flow, Surface Irrigation, Surface Runoff, Wastes, Water Pollution, Water Pollution Sources.

THERMAL POLLUTION OVERVIEW

I INTRODUCTION

Thermal pollution has become an increasingly common term in water management and pollution control circles. The reason is that wasted heat is perpetuating undesirable changes in our aquatic environment, particularly evident in severe cases. In addition, the overall importance of temperature as a water quality parameter has been recognized. We know now that our waterways cannot be viewed or treated as dumpgrounds for waste heat if we desire their continued beneficial usage.

In light of these factors, industry's accelerating emission of heated wastes to the environment poses hazards that have triggered an acute awareness and concern on the part of the public, the policy makers and industry itself. Establishment of State-Federal water quality standards bring the concern into sharp focus. Adding to the problem is the increasing lead time required for delivery of heavy power generating equipment and other industrial machinery, causing hurried management and public policy decisions on plant siting, design and equipment that will have long-range impact on the environment.

II BASIS OF THE PROBLEM

Thermal pollution covers both decreases and increases in normal water temperature; however, almost all cases of thermal pollution involve too much heat, rather than too little.

Many types of human activity can change the normal temperature of water. Temperature changes may be a secondary result, induced by altering the environment (for example, through road building or logging, by creating impoundments, or by diverting flows for irrigation). Or, water temperatures may be changed directly by adding or taking away heat. Our concern over changes in the normal temperatures of water bodies is based primarily on the effect on living organisms. Temperature changes also induce slight variations in physical and chemical properties, thereby affecting such things as gas solubility, particle settling rates, stratification, and chemical rates of reaction. These property changes are usually not too important in themselves but, rather, are significant because of their overall effect on the ecology of a system.

Biological effects of temperature on individual organisms living in water influence all life stages and processes -- reproduction, growth and activity. Most of the effects of increased temperature stem from increased metabolism and activity which require a greater consumption of oxygen. If the oxygen supply is not sufficient, the organism may be subjected to severe stress. The toxicity of certain substances may also raise with temperature increases. Such factors determine the distribution and general well-being of a given organism as well as his basic survival.

Temperature effects on all organisms in an aquatic community are important because of the interdependence of species. The overall thermal environment determines the composition of species which will thrive. As temperatures rise, less desirable species are likely to become established. Continuing productivity of proper food organisms is a basic requirement for harvestable fish, shellfish or other crops. An example of the importance of species composition was observed after start-up of the Chalk Point generating plant. When the water temperature rose due to the plant discharge, extensive beds of Widgeon grass, Ruppia, were replaced by a species of Potamogeton. This was important to the area because the Ruppia had previously been used as food by some 30 species of ducks and birds, but the species which replaced it had very little food value for such waterfowl.

Many industries utilize water for cooling and thereby reject heat to the aquatic environment. In 1964 about 50 billion gallons of water was used for cooling and condensing purposes by the power and manufacturing industries. This was almost one-half of all water used in the United States, or almost one-fifth of our total fresh water supply. Power generating facilities alone account for 80% of this cooling water use.

Because the power industry accounts for such a large portion of cooling water usage, power requirements offer a good correlation to future waste heat loads. Power generation has increased at a net rate of over 7% annually, or, it has doubled every 10 years. This rate of generation is expected to continue into the future. However, waste heat rejection is expected to increase at least as fast as power production! This is because of less power contribution from hydro sources, a limiting efficiency being approached in fossil-fueled plants, and lower efficiency of the new nuclear-fueled plants. Hence, the waste heat load from power plants, on a national basis, will double before 1980 and possibly increase ninefold by the year 2000.

Waste heat from manufacturing will also increase in the future, but the rate of increase will be less than that for power and the total amount of waste heat involved will be much less. Therefore, the primary concern at present is the thermal pollution potential of steam electric power plants, although all industries must be considered.

III CONTROL

The short term solution to waste heat problems can be accomplished through utilization of cooling devices which offer a means of dissipating waste heat under controlled conditions, thereby preventing its undesirable entrance to some water body. The ultimate solution of the problem will be based on new methods of power generation which reject substantially less heat to the environment, and on a management approach which will involve methods of using waste energy for beneficial or constructive purposes.

The setting of Water Quality Standards has done much to speed the development and use of methods and machinery for waste heat control. Standard or "cookbook" approaches in applying control methods are not yet a reality, however, so that each new power plant siting brings its own unique problem.

Two factors which all large, new power plants do have in common are the magnitude and the quality of the waste heat load. It is the combination of low thermal quality and huge quantity which increases the difficulty and cost of heated effluent treatment or use. It is important to emphasize the tremendous amount of cooling water required to operate a single, modern power plant because this factor, by itself, adds to the control problem. Control measures, such as cooling ponds, cooling towers, or dispersion and dilution techniques would be much easier applied to 100 cfs of effluent cooling water than the actual 1000, 1500 or even 2000 cfs which exhaust from plants of 1000 megawatts and more. In most cases once-through cooling is now totally unacceptable because rivers and lakes cannot stand to have significant portions of their water warmed through power plants without causing serious damage to aquatic life.

The efficiency of a power plant naturally affects the amount of waste heat rejected, which is the amount that must be dissipated in any control method. Efficiency, n_t, is the electrical output divided by the thermal input times 100, for percent. For one kilowatt hour, which is equal to 3413 BTU's,

$$n_t = \frac{3413}{3413 + \text{Waste Heat}} \times 100$$

The denominator of the efficiency equation represents the heat required to produce one kilowatt hour of electricity. It is used as a measure of a plant's efficiency, being proportional to efficiency itself, and is called the plant "heat rate."

The heat rate of even the best present day fossil-fueled plants is about 8500 BTU's/KWH. So we see that, even at best, about 5000 BTU's of heat must be wasted for each KWH of electricity that is produced.

We can evaluate more closely the amount of heat rejected to cooling water from a plant, knowing the efficiency or the heat rate and by assuming some reasonable losses. For fossil-fueled plants, losses account for about 15% of the thermal input, which is the heat rate. About 10% goes out the stack; 5% is lost within the plant. So the heat that must be rejected to cooling water, per KWH, is equal to:

$$\text{Heat to cooling water} = 0.85 \times \text{Heat Rate} - 3413$$

Hence, for a fossil-fueled plant at 40% efficiency: (Typical new plant efficiency)

$$\text{Heat Rate} = \frac{3413}{.40} = 8533 \text{ BTU}$$

$$\text{Heat to cooling water} = 0.85\ (8533) - 3413$$

$$= \text{about } 3800 \text{ BTU/KWH}$$

For a nuclear plant, stack losses are not involved and the input losses are estimated to be 5% of the input. Therefore,

$$\text{Heat to cooling water} = 0.95 \times \text{Heat Rate} - 3413$$

So, for a nuclear plant at 33% efficiency, (that's about the maximum for plants planned to 1975):

$$\text{Heat Rate} = \frac{3413}{.33} \quad 10{,}340 \text{ BTU}$$

$$\text{Heat to cooling water} = 0.95\,(10{,}340) - 3413$$
$$= \text{about } 6400 \text{ BTU/KWH}$$

We can see that nuclear plants will not lessen the potential thermal pollution problem. Instead they will reject about 65% more heat to cooling water than fossil-fueled plants.

Today's technology offers practical cooling devices for dissipating waste heat without harm, methods which utilize direct air-water contact depend primarily on evaporation for heat transfer. Each pound of water that is vaporized takes about 1000 BTU's of heat from the water remaining, thereby lowering its temperature.

Cooling ponds are the simplest method for providing air-water contact for cooling heated discharges. A few such ponds or reservoirs are in use at the present time. Cooling ponds can offer a cost advantage, if land is inexpensive, and they may offer recreational benefits. For these reasons cooling ponds warrant further consideration for future use.

The cooling towers which are also used to dissipate heat from a thermal discharge are not new. They have been used extensively in Europe for many years. In this country their primary application has been in small cooling water systems, although some power plants in the Southwest have utilized towers for cooling.

There are many types of towers, but the most common is the wet, or evaporative, type using either mechanical or natural draft inducement. Mechanical draft towers depend upon fans to create air movement through the tower packing, which is the heat transfer section of the tower. The main advantage of the mechanical draft type of tower is that the air flow is relatively constant, regardless of meteorologic conditions. Therefore, tower location is not a critical factor. Also, with controlled air flow, close control of exit cold water temperature may be achieved.

The principal disadvantage to mechanical draft towers is their high operating and maintenance cost, which are incurred because of power requirements and wear in mechanical and electrical components. Another disadvantage is that the humid exhaust is expelled relatively close to the ground -- probably 60 or 70 feet high -- so that fogging or recirculation problems may occur.

The cooling tower which is frequently utilized in large power plant systems at the present time is the wet, natural draft hyperbolic type of tower. Over 20 of these are now in operation or in various stages of construction in this country. Hyperbolic towers derive their name from the profile of the reinforced concrete shell. The largest towers are almost 450 feet high and over 300 feet in diameter at the base. The packing, or heat transfer section, if located in or around the lower 20 to 30 feet of the concrete shell, so most of the shell interior is open space which functions as a stack or chimney. Warm water passing through the packing, heats the air and adds water vapor to it. These processes decrease the air density and cause it to rise up the tower. In operation, a continual upward air movement in the tower is established.

The main reasons for the increased popularity of natural draft towers as compared to other types is their relatively low operating and maintenance costs. They do essentially the same job as mechanical draft towers but without the use of mechanical or electrical components. Also, the great tower heights expel moisture laden air at elevations where they are not likely to result in ground fog or recirculation problems.

Dry towers, those which do not have direct air-water contact, are not in general use for large power plant cooling. They are at a disadvantage, both in terms of performance and economics, when compared to their wet counterparts.

One inherent limitation on dry towers is that the theoretical limit of cooling is governed by the dry bulb temperature. In wet towers this limit is the wet bulb temperature which is usually appreciable lower than dry bulb. Because of heat transfer limitations, dry towers require larger heat transfer surfaces and therefore, overall, the tower is relatively larger.

The cost of using cooling towers as a method of thermal pollution control does not seem prohibitive. For large power plant applications, for example, wet cooling towers have increased production costs in the neighborhood of 5 or 6 per cent. However, in terms of supply cost, or the cost to the consumer, the increase amounts to about one per cent, or slightly more. This is because the increase in production cost is diluted before it gets to the consumer by other costs, including those for transmission, facilities and equipment, and administration.

So in terms of percentage, the cost of preventing thermal pollution is not very much.

There are other methods for preventing or minimizing undesirable temperature conditions. Heated discharges can be manipulated to induce dilution so that heat will be dispersed throughout a larger water mass. In many cases diffusers can be designed to accomplish this type of mixing, which will prevent the occurrence of unallowable water temperatures. The cost of this type of control would generally be less than that for cooling towers, but sufficient flows of dilution water must be available before it can even be considered. Also, the fact that heat is still being added to the water is a disadvantage and may be undesirable enough to warrant a different method of control.

Our present means of controlling thermal pollution depends on treatment; in the future we can look for less abatement and more prevention with respect to waste heat problems -- that is, an evolution to non-treatment control. This can be accomplished in two or three different ways or combination thereof.

First we can look toward increased efficiencies of power generating plants. Nuclear-fueled plants offer much room for improvements in efficiency -- hopefully through the use of different coolants, cycles or reactor design.

We can also look to new methods of power generation which will be highly efficient and not require water for cooling. Use of gas turbines, which operate without cooling water, is increasing at the present time, even though their efficiency is low -- about 25%. It may be possible to raise their efficiency or couple them with other methods of generation to utilize more of the energy, thereby increasing efficiency and reducing cooling water requirements.

Other potential methods for large scale power production include magneto hydrodynamics (MHD) and fuel cells. These do not require cooling water and hold promise of efficiencies greater than modern steam plants.

There are additional new methods which are being investigated, but those mentioned seem to hold the most promise at this time.

Both old and new methods of power production reject energy in the form of waste heat. We are hoping to turn this waste heat into an asset by using it for beneficial purposes. This involves a management approach and the concept of total energy utilization. To implement such an approach, total community energy needs must be analyzed, more efficient methods of energy utilization must be developed for each level of consumption, and constructive uses must be found for waste heat. Investigations are currently underway for valid applications for waste heat in agriculture, aquaculture or industry.

To summarize briefly; there are many real problems associated with waste heat -- its disposal, effects and control. But practical controls are available today. Tomorrow we hope control will be replaced by reduction and utilization of waste heat.

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Descriptors: Heat Pollution, Liquid Wastes, Pollutants, Pollution (Thermal), Power Plants, Thermal Pollution, Waste Water (Pollution), Wastes, Water Cooling, Water Pollution, Water Pollution Sources, Water Temperature

WATER QUALITY SURVEYS

Organizing The Stream Survey

I INTRODUCTION

A Theoretical Approach

1 This discussion will present as a base, the procedures for the conduct of an ideal water quality survey.

B Practical Limitations

1 In practice, limitations of personnel, facilities, time and money, always will require compromise between the ideal and the possible.

C Objectives of Quality Studies

1 The four basic objectives behind the almost limitless number of possible reasons for water quality studies are:

a Determination of the natural water quality of the stream.

b Measurement, in a selected and limited period of time, of the existing effects of wastes on water quality and uses.

c Procurement of data on waste loads, water quality and stream characteristics that will permit projection of the data to describe probable water quality and effects on uses under a variety of conditions other than those that prevailed during the study.

d Determination of corrective measures needed to protect the stream water quality for proper uses.

II PRELIMINARY PLANNING

A Available Information

Assemble and review all readily available maps, information, and reports bearing on the stream under consideration.

B Problem and Objectives of Study

1 Define the problem requiring study as completely as possible on the basis of available information.

C Tentative Plan of Study

1 Prepare a brief preliminary plan of study for guidance in the subsequent field reconnaissance.

2 Include in the preliminary plan:

a Locations and strengths of known sources of waste

b Locations of areas of water use and a list of legitimate water uses

c Section of major stream and locations of important tributaries and unique features such as dams and points of major diversions

d Possible sampling stations

e Sampling frequency and number of samples at each station f Types of laboratory determinations

g Existing stream gauging stations and additional points where stream flow data are needed.

h Other hydrological data needed and possible means of procurement

i Potential laboratory locations

j Special supplies and equipment

k Personnel requirements

l Approximate cost of field operations

III PRELIMINARY FIELD OPERATIONS

A Importance

This step is often neglected, but a small advance party of two or three experienced field men can obtain information and make preparations that will save time and money and avoid much confusion and possible error when the subsequent field study starts.

B Local Contacts

Contact local agencies and individuals who use or have knowledge of the stream, and assemble available maps, reports, operating records, and verbal information.

C Sources of Wastes

1 Sewage

a Obtain best available estimate of sewêred population.

b Examine sewage treatment plant, if any, and obtain copies of operating records on volumes and characteristics of sewage.

c Locate all points of discharge and make any installations necessary for sampling and gauging.

2 Industrial wastes

a Investigate and prepare flow diagram of process with especial reference to points of water use and waste discharge, and quantities of raw materials and finished products.

b Obtain information on plant operating schedule, with especial attention to daily or seasonal variations, and to any anticipated changes in process or increase in production.

c Obtain data on water consumption, numbers of plant employees by work shifts, and disposal of domestic sewage.

d Investigate waste treatment facilities, if any, and obtain operating records on volumes and characteristics of wastes.

e Locate all points of waste discharge, trace origins in plant process, and make any installations necessary for sampling and gauging.

f Collect samples for preliminary examination in laboratory to permit selection of proper analytical procedures.

D Stream

1 Observe entire reach of stream involved by wading, walking bank, or boat.

2 Note especially points of waste discharge and dispersion patterns, visual evidences of pollution confluences and mixing of tributaries, stream flow characteristics, approximate widths and depths, and locations of prospective sampling stations, and of water uses.

3 Collect bottom samples of biological organisms and bottom deposits, and observe evidences of algae.

a Bottom organisms serve as an index of degree of pollution, and of length of river that should be sampled.

b Bottom deposits indicate extent of sludge deposits.

c Algae indicates probable significance of photosynthesis and possible need for night sampling or light-dark bottle technique for full evaluation of dissolved oxygen variations.

4 Determine times of water travel between pertinent points on stream.

a Knowledge of times of water travel not only will permit the most intelligent conduct of study, but also is essential to the complete analysis, interpretation, and projection of data.

b Time and effort involved are a minor portion of the totals devoted to the stream study, and once established, need not be repeated in subsequent studies.

c Observations should be made at three or more stream flows to develop a time of travel versus stream discharge curve, which will permit interpolation for any desired stream flow.

d Methods include: tracing an inherent or added variable stream constituent; timing surface or submerged floats; and determining stream cross sections at selected intervals.

5 Select and identify sampling stations

a For DO evaluation on the majority of streams, there should be: one station just above each major waste source; stations at about half day intervals for two days time of travel below waste sources; and stations at about one day intervals for an additional three to four days time of travel.

b For coliform evaluation, the stations should be similar to those for DO except that the downstream stations at one day intervals should extend to a total of 10 to 20 days time of water travel below the source of wastes.

c In very small or very turbulent streams, natural purification may be well advanced in as little as one to two days time of water travel and stations should be at much closer intervals than suggested above.

d If tributary streams are important, there should be one station near the mouth of each tributary and one on the main stream just above each confluence.

e Stations should be established as close as practicable to points or areas of important water uses.

f At stations where wastes and tributary waters are well mixed, one sampling point near mid-channel usually is adequate.

g At stations where mixing is inadequate, common practice is to sample at the quarter points of the stream.

h Identify stations by adequate description and marking, and make any necessary preparations for use by sampler.

i Select best access routes to sampling stations and make a round-trip to stations to determine time required for sampling.

E Preliminary Stream Samples

1 Collect one or more sets of stream samples for preliminary examination.

a Dissolved oxygen results will be useful in establishing sampling stations and length of stream that should be studied b Determinations of coliform organisms and BOD will assist in selection of proper dilutions for subsequent use when study is started.

c Determinations of other constituents will enable analyst to select most acceptable laboratory procedures and to determine sizes of sample portions necessary for those analyses in which concentrations of constituents govern choice of portion sizes.

F Laboratory Location

Examine potential laboratory locations in relation to convenience to the field operations, adequacy of space and utilities, storage space, and revisions or additional facilities that will be needed.

G Miscellaneous

1 Locate local sources of supplies that will be purchased during study.

2 Determine transportation routes and schedules for shipments of samples, supplies, and materials.

3 Arrange for local personnel and special facilities that will be used.

4 Investigate rooming facilities convenient to the field operations.

IV FINAL PLANNING

A Revision of Preliminary Plan

With the preliminary plan as a guide, and with the knowledge gained from the preliminary field operations it will be possible to prepare an intelligent, workable plan for the field study.

B Objectives of Study

Redefine, add to, or delete from, the initial list of objectives, and prepare a set of specific objectives which will include provisions for all essential answers to the problem at hand, but will also eliminate needless expenditure, on nonessential matters, of effort which might better be devoted to the principal problem.

C Period of Field Operations

1 The overall period selected for the study should be at the time of year when past experience indicates that streamflows usually are relatively stable.

2 For evaluation of DO depletion; a period of warm weather and low streamflow is desirable.

3 For evaluation of coliform contamination, a period of intermediate flow may be desirable.

4 A period of intensive round-the-clock sampling for a few days generally is preferable to a period of a month of sampling daily or on alternate days, but a combination of both methods may be better than either one.

D Sampling

1 General

a If sources of wastes do not vary significantly during period of study, the wastes may be sampled in advance of the stream study.

b Sampling procedures and preservation of samples should follow "Standard Methods for the Examination of Water and Wastewater" and/or procedures recommended by the investigators agency.

2 Sewage

a Collect samples around-the-clock at 15 minute to 1 hour intervals for three to seven days.

b Composite sample portions in portion to flow for three to four equal time periods of each day.

3 Industrial wastes

a In general, the better known the process is, and the less variable the waste discharge, the less detailed need the sampling be.

b For well known processes with little variation, equal sample portions collected at one-half to one hour intervals and composited for eight hours or for a complete cycle of operation on three to five days should be adequate.

c For little known and variable processes, detailed and prolonged sampling may be necessary, with samples collected at 5 to 15 minute intervals, and composited in proportion to flow for six to 24 hour periods for seven or more days or complete cycles of operation.

4 Stream

a If streamflow, waste discharge, or oxygen production by algae vary widely throughout the day, sample around-the-clock at several stations for at least one or two days to establish the daily cycle of variation in waste constituents and effects.

b In most streams, grab samples each day or on alternate days for two weeks to a month is adequate if conditions remain stable.

c The total number of samples collected from a single station during a field study generally ranges between 15 and 20. In the absence of wide variations in streamflow, 12 to 15 sets of samples usually yield adequate, usable results.

d If sampling is not around the clock, sample collection should be varied throughout the day as much as is feasible, and as a minimum, the direction of the sampling trip should be reversed on alternate days.

e When more than one point is sampled at a station, individual field determinations are made and a separate bacteriological sample is taken at each point, but samples for other determinations may be combined in a single composite. Bacteriological samples from two or more points at the same station may be composited in the laboratory.

E Gauging

1 Methods of waste and stream gauging are covered in another reference outline.

2 Generally stream discharge records are obtained from existing U. S. Geological Survey stations, or from special stations established and rated by them upon request.

3 Streamflow data at each main stream sampling station generally can be computed with sufficient accuracy from records for one or two main stream gauging stations and one for each major tributary.

F Laboratory Operations

1 Select principal determinations that will measure pertinent waste constituents and their effects, and auxilliary determinations that will contribute to interpretation of principal results.

2 Reject determinations that would be "interesting" but would not contribute to solution of the problem.

3 Generally useful principal determinations common to many stream studies are those for coliform bacteria, bottom organisms, temperature, DO, and BOD, and auxiliary determinations frequently are for pH, alkalinity, and turbidity.

Other determinations must be selected for special purposes and for special types of wastes.

5 Laboratory methods should follow procedures recommended in "Standard Methods for the Examination of Water and Wastewater."

6 Avoid excessive overload of the laboratory by working closely with the

laboratory supervisor during the planning stage and accepting his estimate of the volume of work that can be handled.

7 In establishing the laboratory work load, allow time for calculation of analytical results at the end of each day.

8 Ship samples to the headquarters laboratory for all determinations of stable or preserved constituents that do not have to be made in the field.

G Personnel

1 The field crew should possess competencies for the specific tasks involved, or should be trained in advance if necessary.

2 Personnel needed commonly will include:

a Sanitary engineers

b Chemists

c Bacteriologists

d Biologists

e Sampling and laboratory aides

H Supplies and Equipment

1 Prepare and use a check list of needed field supplies and equipment.

2 All operations that can be performed in advance at headquarters, such as training, purchases, equipment repair, and reagent preparation will save money and valuable time in the field.

I Cost Estimate

1 Revise the initial estimate of cost of field operations in light of the final plan adopted.

2 Adjust the final plan to fit the money available for the study, or arrange for the additional money needed.

V FIELD OPERATIONS

A Briefing

1 Assemble the field crew and explain the problem, objectives, and details of field operations.

2 Take at least key personnel on a tour of pertinent physical features of the study.

3 Specify responsibilities of individuals for the various phases of the operation.

B Communications

Arrange a system of communication by which any member of the crew can be contacted within a reasonable length of time.

C Records

1 A few simple field and laboratory forms will serve to systematize the maintenance of basic records.

2 Keep all permanent field and laboratory records in bound volumes.

3 Encourage the recording of other than routine observations by both sampling and laboratory personnel.

4 Write down at once, and do not depend on memory for all significant observations.

D Revision of Operations

1 Review all daily data accumulated at the end of each day, and note especially any omissions or unusual results.

2 On the basis of the daily review, consider the need to:

a Add new determinations if needed.

b Omit determinations that are not showing significant results.

c Revise analytical methods.

d Change sampling stations or scheduled times of sampling.

e Investigate causes of apparently abnormal or erroneous data.

3 Do not revise operations unless the data or other new information indicates changes are essential to achieve the desired objectives.

VI USE OF DATA

A Analysis and Interpretation

1 The raw data are of only limited value until submitted to analysis and interpretation by an experienced person who is familiar with the problem involved.

2 Methods of analysis and interpretation constitute a major subject in themselves and are covered by other lectures.

3 No amount of statistical manipulation of the data can produce sound conclusions unless the stream study is soundly conceived, and carefully and conscientiously executed.

B Report

A report should be prepared if the data are to achieve their maximum usefulness, since raw data in dead files only rarely benefit anyone.

C Ultimate Disposal

After use, the data should be filed, indexed, and describ d so that they can be available and understandable years later to others not directly associated with the study.

D Maximum Usefulness

The results of a stream pollution study yield their maximum benefit and return their greatest satisfaction to those who worked to obtain them only when the results serve as a basis for actual correction of an abuse of stream water quality.

REFERENCE

Kittrell, F.W. A Practical Guide to to Water Quality Studies of Streams. USDI, FWPCA. CWR-5. 1969.

This outline was prepared by Francis W. Kittrell, former Special Consultant, National Field Investigations Center, --Cincinnati Office of Enforcement and General Counsel, EPA, Cincinnati, OH 45268

Descriptors: Baseline Studies, Data Collections, Investigations, Hydrologic Data, Methodology, Natural Streams, On-Site Data Collections, On-Site Investigations Surveys, Planning, Sampling, Stream Pollution, Wastes, Water Analysis, Water Quality

WATER QUALITY SURVEYS

Organizing The Lake or Impoundment Survey

I INTRODUCTION

Lake sampling and data collection are influenced by physical features, chemical factors, and biological communities. Physical features include water temperature, turbidity, color, water movement, light penetration, wind velocity and direction, bottom deposits, and size, shape and slope of the lake basin. Chemical factors include alkalinity, pH, dissolved oxygen, free carbon dioxide, hardness, nitrogen (organic, ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen), phosphate (total and soluble), as well as other specific elements that may be of interest in a particular problem. Biological communities include the littoral community composed of rooted vegetation, attached algae, fish, and a host of invertebrates; the limnetic community, principally fish and plankton; and the benthic community of midge larvae, sludgeworms, fingernail clams, and other bottom dwelling organisms.

II PROBLEM SOLVING

A Development of Objectives

B Thorough Field Investigation

C Precise Recommendations Based on Factual Findings

D Prediction of water quality:

1 If recommendations are not met

2 When recommendations are met

3 If recommendations are only partially met

E Demonstration to prove authenticity of predictions

III PRELIMINARY SURVEY JUDGMENTS

A Define specifically the problem; develop objectives.

B Determine the types of samples necessary to point to a solution to the problem.

C Delineate the sampling points for each of the selected types of samples.

D Select the time, and periodicity to sample.

E Judge the number of samples necessary to complete the study.

1 Define specifically the problem. (Let us assume that the problem can be defined as one of nuisance algal and aquatic weed growths, nuisance midge fly production and occasional shoreline odors.)

2 Determine the types of samples necessary to point to a solution to the problem. These include:

a Flow measurements on streams influent to and discharging from the lake or reservoir.

b Flow measurements on suspected municipal and industrial waste sources and from the receiving streams at points of waste discharge.

c Water samples for selected chemical determinations at flow measurement points, from stream reaches known to be unaffected by suspected waste sources, from area streams carrying natural land drainage only, from precipitation during study period, and from area shallow wells.

d Conduct a house to house survey of shoreline dwellings to determine method of waste disposal, number of residences, and period of yearly occupancy.

e Determine from county agents the types and amounts of fertilizers applied to the drainage basin and the application time for the crops produced.

f Conduct a limnological study of the lake or impoundment:

1) Determine certain morphometric characteristics including area, mean depth, maximum depth, area of depth zones, volume of depth strata, shore length, area of islands, shore length of islands, drainage area, and rate of runoff.

2) Collect water samples for selected chemical determinations at predetermined points, and vertically within the water body. Vertical temperature and dissolved oxygen determinations are necessary.

3) Collect water samples for bacteriological determination from predetermined points, especially those areas subject to water-contact recreational sports such as swimming and skiing.

4) Collect plankton samples, determine percent predominant species composition, estimate average standing crop.

5) Map, identify, and estimate abundance of the submerged aquatic vegetation population.

6) Determine average standing crop of bottom fauna and determine percent composition of major benthic forms.

7) Estimate standing crop of fishes, determine predominant species, determine relative growth rates.

3 Delineate the sampling points for each of the types of samples to be collected. (Many times the limnological samples from the lake are collected from imaginary transection lines.)

4 Select the time, season, and periodicity of sample collections.

5 Judge the number of samples necessary to complete the study.

a To ensure a well-organized survey and laboratory operation, consideration should be given to estimating the total number of samples to be collected during each phase of the study. A form such as the one that follows is often used.

IV SURVEY ORGANIZATION

A Determine location of laboratory.

1 Field unit, or

2 Permanent station

B Determine method of sample handling, preservation if any, and transportation of samples to laboratory.

C Select survey teams and assign duties.

1 Flow measurement team

2 Stream sample collectors

3 Biological team

D Keep collected data current, understandable, and available to all participants.

REQUEST FOR LABORATORY SERVICES

DATE________________

SURVEY________________________ PROJECT CHIEF________________

LABORATORY LOCATION________________________________

FIELD CONTACT: NAME________________________________

ADDRESS________________________________

____

PHONE____________________

SAMPLING DATES____________________TO____________________

ANALYSES REQUIRED

CHEMICAL	ESTIMATED SAMPLES/DAY	TOTAL SAMPLES

BACTERIOLOGICAL	ESTIMATED SAMPLES/DAY	TOTAL SAMPLES

BIOLOGICAL - TYPES OF SAMPLING	TOTAL SAMPLING LOCATIONS
PLANKTON	
BENTHIC	
FISH	
OTHER	

REMARKS

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This outline was prepared by K.M. Mackenthun, Formerly, Acting Chief, Biological and Chemical Section, Technical Advisory and Investigations Branch, Division of Technical Services, Federal Water Pollution Control Administration.

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FLOW MEASUREMENT

I INTRODUCTION

Flow measurements are among the more important data collected during a water quality survey. Such measurements are used to interpret data variations, calculate loadings, and expedite survey planning. If the analysis of survey data involves estimation of loads, the accurate measurement of discharge assumes a level of importance equal to that of laboratory and analytical results.

In the following discussion, procedures for measurement of stream flow and waste discharge are described. Some of these procedures are used in long-term, very detailed water quality and supply studies; others are more suited to short-term pollution surveys.

II PLANNING

A Station Location

Four factors influence location of gauging or flow measurement stations:

1 Survey objectives

2 Physical accessibility

3 Characteristics of the stream bed

4 Hydrologic effects

Survey objectives represent the major influence on station location; depending upon objectives, gauging stations may be located above and/or below confluences and outfalls.

Physical accessibility determines the ease and cost of installation and maintenance of the station. The characteristics of the stream bed may greatly influence the obtainable accuracy of measurement. For instance, rocky bottoms greatly reduce the accuracy of current meters. Sedimentation in pools behind control structures may influence stage-discharge relationships. Hydrologic variations in stream flow may cause washout or bypass of the gauging station. In the Southwest, flash floods have been known to wash out or bypass gauging stations by assuming different channels of flow.

B Methodology

Choice of a specific measurement procedure is dependent upon at least three considerations:

1 The relation between obtainable and desired accuracy

2 Overall cost of measurement

3 The quantity of flow to be measured

Ideally, discharge measurements should be reported to a specific degree of accuracy; the gauging procedure greatly influences this accuracy. The influence of overall cost on the gauging program is readily apparent. Extensive, detailed studies are usually characterized by high costs for automatic instrumentation and low personnel cost; the opposite is usually true for less detailed studies. The range of flows to be measured (within acceptable accuracy) is, of course, not known prior to the survey. However, experienced personnel usually can make reasonable estimates of expected flows from visual observations and other data, and may recommend appropriate gauging procedures. In this regard, experienced personnel always should be consulted.

III MEASUREMENT

A Streams, Rivers, and Open Channels

1 Current Meter

The current meter is a device for measuring the velocity of a flowing body of water. The stream cross section is divided into a number of smaller sections, and the average velocity in each section is determined. The discharge is then found by summing the products of area and velocity of each section.

2 Stage-discharge relationships

Large flows usually are measured by development of and reference to a stage-discharge curve; this procedure has long been used by the U. S. Geological Survey. Such gauging stations are composed of a control structure located downstream of the location of measurement and some type of water level indicator which identifies the height of the water surface above a previously determined datum.

Location of the control structure so that reliable measurements of flow will be obtained at all river stages is particularly important. The water level may be continuously recorded by an automatic recorder located in a wet well or may be indicated directly on a staff gauge located at the bank of the river. Such stations must be calibrated by measurement of flow by velocity-area methods (current meter) at all expected stages of river flow.

3 Weirs

A weir may be defined as a dam or impediment to flow, over which the discharge conforms to an equation. The edge or top surface over which the liquid flows is called the weir crest. The sheet of liquid falling over the weir is called the nappe. The difference in elevation between the crest and the liquid surface at a specified location, usually a point upstream, is called the weir head. Head-discharge equations based on precise installation requirements have been developed for each type of weir. Weirs so installed are called standard weirs. Equations for non-standard installations or unusual types may be derived empirically.

Weirs are simple, reliable measurement devices and have been investigated extensively in controlled experiments. They are usually installed to obtain continuous or semi-continuous records of discharge. Limitations of weirs include difficulty during installation, potential siltation in the weir pond, and a relatively high head requirement, 0.4 - 2.0 feet. Frequent errors in weir installation include insufficient attention to standard installation requirements and failure to assure completely free discharge of the nappe.

a Standard suppressed rectangular weir

This type of weir is essentially a dam placed across a channel. The height of the crest is so controlled that construction of the nappe in the vertical direction is fully developed. Since the ends of the weir are coincident with the sides of the channel lateral contraction is impossible. This weir requires a channel of rectangular cross section, other special installation conditions, and is rarely used in plant survey work. It is more commonly used to measure the discharge of small streams.

The standard equation for discharge of a suppressed rectangular weir (Francis equation) is:

$$Q = 3.33\ LH^{3/2}$$

where

Q = discharge, cfs
L = length of the weir crest, feet
H = weir head, feet

The performance of this type of weir has been experimentally investigated more intensively than that of other weirs. At least six forms of the discharge equation are commonly employed. The standard suppressed weir is sometimes used when data must be unusually reliable.

b Standard contracted rectangular weir

The crest of this type of weir is shaped like a rectangular notch. The sides and level edge of the crest are so removed from the sides and bottom of the channel that contraction of the nappe is fully developed in all directions. This weir is commonly used in both plant surveys and measurement of stream discharge.

The standard equation for discharge of a contracted rectangular weir (corrected Francis equation) is

$$Q = 3.33\ (L - 0.2H)H^{3/2}$$

where

Q = discharge, cfs
L = length of the level crest edge, feet
H = weir head, feet
$0.2H$ = correction for end contractions as proposed by Francis

c Cipolletti weir

The Cipolletti weir is similar to the contracted rectangular weir except that the sides of the weir notch are inclined outward at a slope of 1 horizontal to 4 vertical. Discharge through a Cipolletti weir occurs as though end contractions were absent and the standard equation does not include a corresponding factor for correction.

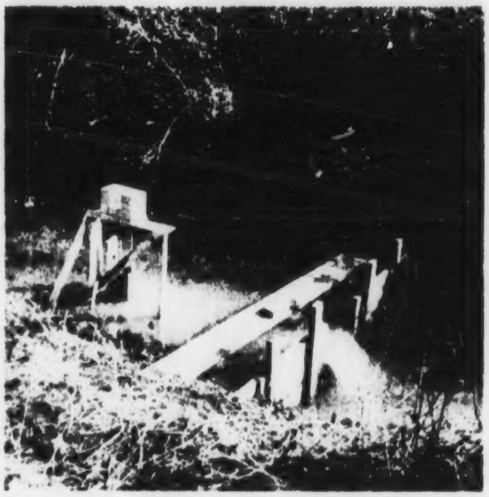


Figure 1. STANDARD CONTRACTED RECTANGULAR WEIR

The standard equation for discharge through a Cipolletti weir is

$$Q = 3.367\ LH^{3/2}$$

where

Q = discharge, cfs

L = length of the level crest edge, feet

H = weir head, feet

The discharge of a Cipolletti weir exceeds that of a suppressed rectangular weir of equal crest length by approximately 1 percent.

d Triangular weirs

The crest of a triangular weir is shaped like a V-notch with sides equally inclined from the vertical. The central angle of the notch is normally 60 or 90 degrees. Since the triangular weir develops more head at a given discharge than does a rectangular shape, it is especially useful for measurement of small or varying flow. It is preferred for discharges less than 1 cfs, is as accurate as other shapes up to 10 cfs, and is commonly used in plant surveys.

The standard equation for discharge of a 90° triangular weir (Cone formula) is

$$Q = 2.49H^{2.48}$$

where

Q = discharge, cfs
H = weir head, feet

Crest height and head are measured to and from the point of the notch, respectively.

e Accuracy and installation requirements

Quotations of weir accuracy express the difference in performance between two purportedly identical weirs and do not include the effects of random error in measurement of head. Weirs installed according to the following specifications should measure discharge within ± 5% of the values observed when the previously cited standard equations were developed.

1) The upstream face of the bulkhead and/or weir plate shall be smooth and in a vertical plane perpendicular to the axis of the channel.

2) The crest edge shall be level, shall have a square upstream corner, and shall not exceed 0.08 in (2 mm) in thickness. If the weir plate is thicker than the prescribed crest thickness the downstream corner of the crest shall be relieved by a 45° champfer.

3) The pressure under the nappe shall be atmospheric. The maximum water surface in the downstream channel shall be at least 0.2 ft. below the weir crest. Vents shall be provided at the ends of standard suppressed weirs to admit air to the space beneath the nappe.

4) The approach channel shall be straight and of uniform cross section for a distance above the weir of 15 to 20 times the maximum head, or shall be so baffled that a normal distribution of velocities exists in the flow approaching the crest and the water surface at the point of head measurement is free of disturbances. The cross-sectional area of the approach channel shall be at least 6 times the maximum area of the nappe at the crest.

5) The height of the crest above the bottom of the approach channel shall be at least twice, and preferably 3 times, the maximum head and not less than 1 foot. For the standard suppressed weir the crest height shall be 5 times the maximum head. The height of triangular weirs shall be measured from the channel bottom to the point of the notch.

6) There shall be a clearance of at least 3 times the maximum head between the sides of the channel and the intersection of the maximum water surface with the sides of the weir notch.

7) For standard rectangular suppressed, rectangular contracted, and Cipolletti weirs the maximum head shall not exceed 1/3 the length of the level crest edge.

8) The head on the weir shall be taken as the difference in elevation between the crest and the water surface at a point upstream a distance of 4 to 10 times the maximum head or a minimum of 6 feet.

9) The head used to compute discharge shall be the mean of at least 10 separate measurements taken at equal intervals. The head range of the measuring device shall be 0.2 - 1.5 feet.

The capacities of weirs which conform to these specifications are indicated in Table 1.

4 Parshall flume

The Parshall flume is an open constricted channel in which the rate of flow is related to the upstream head or to the difference between upstream and downstream heads. It consists of an entrance section with converging vertical walls and level floor, a throat section with parallel walls and floor declining downstream, and an exit section with diverging walls and floor inclining downstream. Plan and sectional views are shown in Figure 2. Advantages of the Parshall flume include a low head requirement, dependable accuracy, large capacity range, and self cleaning capability. Its primary disadvantage is the high cost of fabrication; this cost may be avoided by use of a prefabricated flume. Use of prefabricated flumes during plant surveys is becoming increasingly popular.

a Standard equations

The dimensions of Parshall flumes are specified to insure agreement with standard equations. Table of dimensions are available from several sources [3, 4]. For flumes of 6 inch to 8 foot throat width the following standard equations have been developed.

1) 6 inch throat width

$$Q = 2.06 \; H_a^{1.58}$$

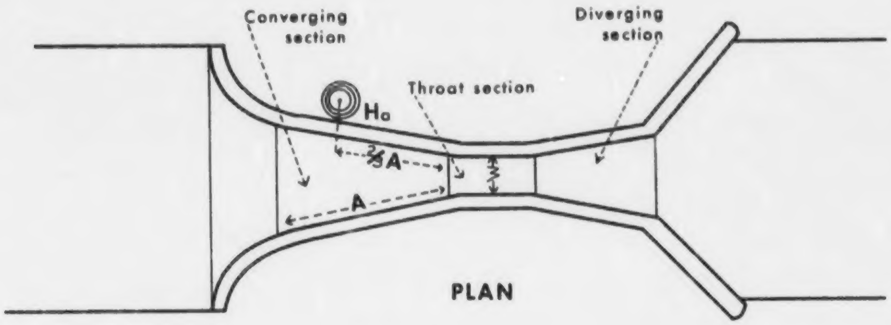
2) 9 inch throat width

$$Q = 3.07 \; H_a^{1.53}$$

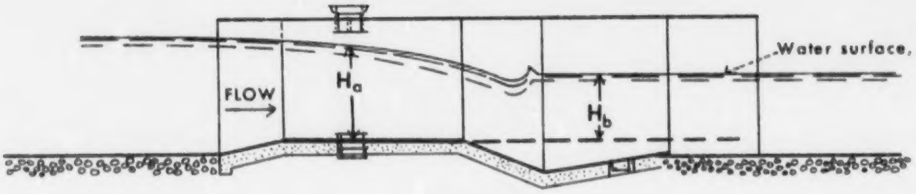
TABLE 1 DISCHARGE OF STANDARD WEIRS

Crest Length (Feet)	Contracted Rectangular* Weir (discharge-cfs)		Suppressed Rectangular* Weir (discharge-cfs)		Cipolletti* Weir (discharge-cfs)		90° Triangular* Weir (discharge-cfs)	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1.0	.590	.286	.631	.298	.638	.301		
1.5	1.65	.435	1.77	.447	1.79	.452		
2.0	3.34	.584	3.65	.596	3.69	.602		
2.5	5.87	.732	6.30	.744	6.37	.753		
3.0	9.32	.881	10.0	.893	10.1	.903		
3.5	13.8	1.03	14.8	1.04	15.0	1.05		
4.0	19.1	1.18	20.4	1.19	20.6	1.20		
4.5	25.6	1.33	27.5	1.34	27.8	1.35		
							6.55	.046
5.0	28.8	1.48	30.6	1.49	30.9	1.51		
6.0	34.9	1.78	36.7	1.79	37.1	1.81		
7.0	41.0	2.07	42.8	2.08	43.3	2.11		
8.0	47.1	2.37	48.9	2.38	49.5	2.41		
9.0	53.2	2.67	55.0	2.68	55.7	2.71		
10.0	59.3	2.97	61.1	2.98	62.0	3.01		

* $H \geq 0.2$ ft, $H \leq 1.5$ ft, $H \leq 1/3\ L$


Converging section
Diverging section
Throat section
H_a
$\frac{2}{3}A$
A

PLAN


Water surface, a
FLOW
H_a
H_b

SECTION

FIGURE 2 PARSHALL FLUME

3) 1 to 8 foot throat width

$$Q = 4WH_a^{1.522W^{0.026}}$$

where

Q = free-flow discharge, defined as that condition which exists when the elevation of the downstream water surface above the crest, H_b, does not exceed a prescribed percentage of the upstream depth above the crest, H_a. The prescribed percentage of submergence is 60 percent for 6 and 9 inch flumes and 70 percent for 1 to 8 foot flumes

W = throat width, feet

H_a = upstream head above the flume crest

b Head loss

The head required by a Parshall flume is greater than $(H_a - H_b)$ because H_a is measured at a point in the converging section where the water surface has already begun to decline. Table 2 indicates the total head requirements of standard Parshall flumes. These losses should be added to the normal channel depth to determine the elevation of the water surface at the flume entrance. No head losses are indicated for discharge-throat width combinations for which H_a is less than 0.2 ft. or greater than 2/3 the sidewall depth in the converging section.

c Accuracy and installation requirements

A Parshall flume will measure discharge within ± 5% of the standard value if the following conditions are observed.

1) The dimensions of the flume shall conform to standard specifications.

2) The downstream head, H_b, shall not exceed the recommended percentage of the upstream head, H_a.

TABLE 2 HEAD LOSS IN STANDARD PARSHALL FLUMES UNDER FREE DISCHARGE

Discharge (cfs)	Head Loss, Feet, in Flume of Indicated Width							
	1 foot	2 feet	3 feet	4 feet	5 feet	6 feet	7 feet	8 feet
0.5	.08							
1.0	.14	.09	.06					
2.5	.26	.16	.12	.10	.08	.07	.06	.05
5.0	.42	.27	.20	.16	.13	.12	.10	.09
10.0	.70	.45	.34	.27	.22	.19	.17	.15
30.0			.70	.56	.47	.40	.35	.30
50.0					.68	.57	.49	.41

$H_a \geq 0.2$, $H_a \leq 2.0$

3) The upstream head shall be measured in a stilling well connected to the flume by a pipe approximately 1-1/2 inches in diameter.

4) The flume shall be installed in a straight channel with the centerline of the flume parallel to the direction of flow.

5) The flume shall be so chosen, installed, or baffled that a normal distribution of velocities exists at the flume entrance.

5 Tracer materials

Techniques, materials, and instruments are presently being refined to permit accurate measurement of instantaneous or steady flow with several tracer materials. Measurements are made by one of two methods:

a Continuous addition of tracer

b Slug injection

With the first method, tracer is injected into a stream at a continuous and uniform rate; with the second a single dose of tracer material is added. Both methods depend on good transverse mixing and uniform dispersion throughout a stream. The concentration of tracer material is measured downstream from the point of addition. When continuous addition is employed, flow rates are calculated from the equation:

$$q \cdot C = (Q + q)\, c$$

in which q = rate of tracer addition to the stream at concentration, C, Q = the stream flow rate, and c = the resulting concentration of the stream flow combined with the tracer. For the slug injection method

$$Q = \frac{S}{\bar{c}\ \Delta t}$$

in which Q = the stream discharge, S = the quantity of tracer added, $\bar{c}$ = the weighted average concentration of tracer material during its passage past the sampling point, and Δt = the total time of the sampling period. Disadvantages of tracer methods include mixing, natural adsorption and interference, and high equipment costs.

6 Floats

Floats may be used to estimate the time of travel between two points a known distance apart. The velocity so obtained may be multiplied by 0.85 to give the average velocity in the vertical. Knowing the mean velocity and the area of the flowing stream, the discharge may be estimated. Floats should be employed only when other methods are impractical.

B Pipes and Conduits

1 Weirs and Parshall flumes

Weirs and Parshall flumes are commonly installed in manholes and junction boxes and at outfalls to measure flow in pipes. All conditions required for measurement of open channel flow must be observed.

2 Tracer materials

These methods are popular for measurement of pipe flow because they do not require installation of equipment or modification of the flow. These are especially convenient for measurement of exfiltration and infiltration.

3 Depth-slope

If the depth of the flowing stream and the slope of the sewer invert are known, the discharge may be computed by means of any one of several formulas.

a Manning formula

$$Q = \frac{1.486}{n}\ A\, R^{2/3} S^{1/2}$$

where

Q = discharge, cfs

n = roughness coefficient

A = area of flow, sq. ft.

R = hydraulic radius, ft.

= area divided by wetted perimeter, ft.

S = slope, ft. per ft.

b Chezy formula

$$Q = CA\sqrt{RS}$$

where

Q = discharge, cfs

C = friction coefficient

A = area of flow, sq. ft.

R = hydraulic radius, ft.

= area divided by wetted perimeter, ft.

S = slope, ft. per ft.

4 Open end pipe flow

The following methods can be employed when other more precise means are not practical. They can be employed, however, only when there is free discharge to the air.

a Coordinate method (Figures 3, 4, and 5)

Discharge may be computed by the following formula:

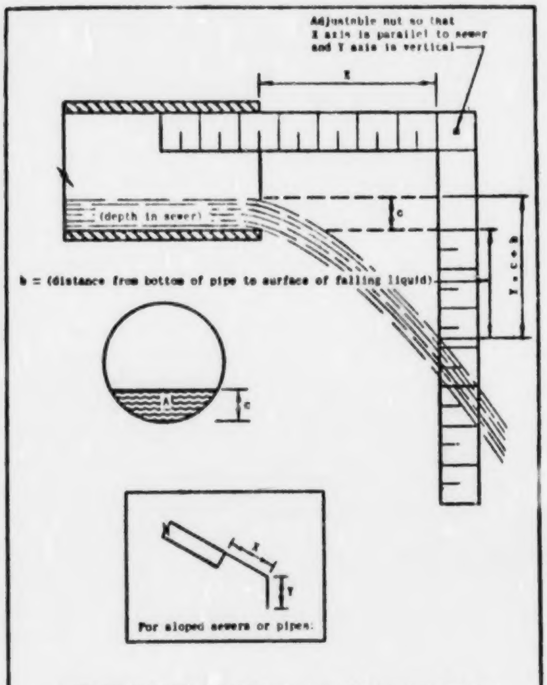
$$Q\ (\text{gpm}) = \frac{1800\ AX}{Y}$$

where

A = cross sectional area of liquid in the pipe (sq. ft.)

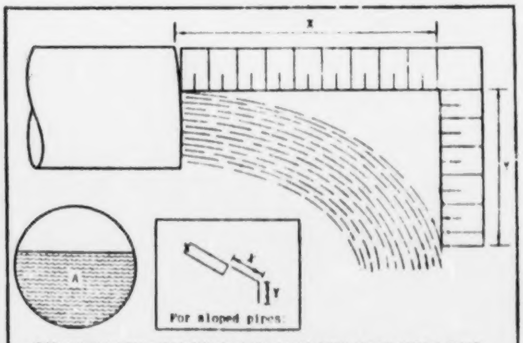
X = distance between the end of the pipe and the vertical gauge in ft., measured parallel to the pipe.

Y = vertical distance from water surface at the end of the pipe to the intersection of the water surface with the vertical gauge, in ft.


Adjustable nut so that
X axis is parallel to sewer
and Y axis is vertical
X
(depth in sewer)
c
b = (distance from bottom of pipe to surface of falling liquid)
Y = c + b
A
c
X
Y
For sloped sewers or pipes:

OPEN-PIPE FLOW MEASUREMENT - THIS DEVICE, ADJUSTED TO THE SLOPE OF A SEWER AND CALIBRATED, CAN THEN BE CLAMPED TO THE SEWER OUTFALL.

Figure 3


X
Y
A
X
Y
For sloped pipes:

OPEN-PIPE FLOW MEASUREMENT REQUIRES TWO DIMENSIONS THAT LOCATE THE SURFACE OF STREAM AFTER IT LEAVES THE PIPE.

Figure 4

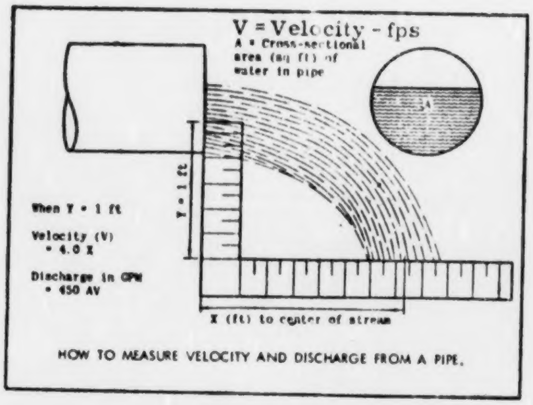

V = Velocity - fps
A = Cross-sectional area (sq ft) of water in pipe
A
Y = 1 ft
When Y = 1 ft
Velocity (V) = 4.0 X
Discharge in GPM = 450 AV
X (ft) to center of stream
HOW TO MEASURE VELOCITY AND DISCHARGE FROM A PIPE.

Figure 5

b California pipe flow method (Figure 6)

This method may be used only for horizontal pipes having free discharge. If the pipe is not horizontal, a connection must be made to one that is. The horizontal length must be not less than 6 times the diameter of the pipe. Discharge may be computed by the following formula:

$$Q \text{ (gpm)} = T \times W$$

$$\text{where } T = 3{,}900 \left(1 - \frac{a}{d}\right)^{1.88}$$

$$W = d^{2.48}$$

where a and d are measured in feet

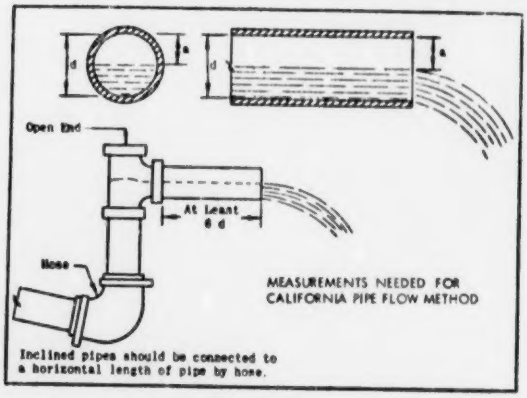

d
a
d
a
Open End
At Least 6 d
Hose
MEASUREMENTS NEEDED FOR CALIFORNIA PIPE FLOW METHOD
Inclined pipes should be connected to a horizontal length of pipe by hose.

Figure 6

C Head Measuring Devices

Several of the above gauging methods require the measurement of water level in order that discharge may be determined. Any device used for this purpose must be referenced to some zero elevation. For example, the zero elevation for weir measurements is the elevation of the weir crest. The choice of method is dependent upon the degree of accuracy and the type of record desired.

1 Hook gauge

The hook gauge measures water elevation from a fixed point. The hook is dropped below the water surface and then raised until the point of the hook just breaks the surface. This method probably will give the most precise results when properly applied.

2 Staff gauge

The staff gauge is merely a graduated scale placed in the water so that elevation may be read directly.

3 Plumb line

This method involves measurement of the distance from a fixed reference point to the water surface, by dropping a plumb line until it just touches the water surface.

4 Water level recorder

This instrument is used when a continuous record of water level is desired. A float and counterweight are connected by a steel tape which passes over a pulley. The float should be placed in a stilling well. A change in water level causes the pulley to rotate which, through a gearing system, moves a pen. The pen traces water level on a chart which is attached to a drum that is rotated by a clock mechanism. When properly installed and maintained, the water level recorder will provide an accurate, continuous record.

ACKNOWLEDGMENT:

Certain portions of this outline contains training material from prior outlines by P.E. Langdon, A.E. Becher, and P.F. Atkins, Jr.

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Descriptors: Chezy Equation, Discharge Measurement, Discharge (Water), Flow, Flow Measurement, Flow Rates, Flumes, Mannings Equation, Open Channel Flow, Pipe Flow, Streamflow, Venturi Flumes, Water Flow, Water Level Recorders, Weirs

TRACING NATURAL WATERS

I INTRODUCTION

The validity of water pollution studies is dependent upon an ability to describe the actual time and space situation of a pollutant as it blends with the natural receiving water. This understanding is important in order to predict the subsequent effects of the pollutant on the receiving water and subsequent users or to detect the sources of pollution.

The nature of this blending is dependent upon the physical and chemical properties of both the pollutant and the receiving water as well as the mixing and flow characteristics of the receiving channel, basin, or aquifer. Tracing is an attempt to approximate the actual motion and mixing of this blend as it moves through the channel, basin or aquifer.

Although there has been scant reference to tracers in texts there has been rapidly increasing number of technical articles describing their use in recent years. A comprehensive compilation of early radioactive tracer studies may be found in the report of the "Time of Flow Studies, Ottawa River, Lima, Ohio"(1) one of the early attempts to compare various tracer techniques under similar field conditions. Several reports (see Bibliography) have discussed the use of dyes during the early 1960's.

More refined and extensive tracer investigations may be expected as experience is gained and as more complex studies arise. A water quality agency should develop competence in at least one tracer technique and would be wise to be capable in some others as well.

II PURPOSES

Tracer applications are still in a highly developmental stage. One finds many words or phrases to suggest similar field findings. In broad terms these measurements are concerned with indications of mass movement, blend mixing, and flow direction. Basically, tracers are used to determine:

A Flow Rates

1 In freshwater channels or lakes such terms as passage time, time of travel, time of flow, flow quantity or flow volume are used.

2 In estuaries such terms as rate of renewal, flushing rate, drift velocity, mass transfer or net (tidal) drift are used. The tidal fluctuation imposes a need for consideration of flow reversal effects.

3 In subsurface waters such terms as recharge rate, flow rate and residence time are used. Related factors such as basin capacity, porosity and permeability are determined.

B Flow Direction

1 In estuaries

a Direction of flow relative to tidal phases which do not necessarily correspond to slack conditions.

b Obscure current circulation as influenced by the interlated factors of tides, winds, Coriolis forces, topography and density gradients.

2 In ground water

a Aquifer may flow contrary to initial superficial impression.

b Hydrostatic conditions may change and reverse normal flow direction.

C Mixing Patterns

1 Short circuit effects

a Eddying - primarily the result of surface channel and basin configuration.

b Stratification - primarily due to temperature and density differences.

c Inter-connections - between aquifers in ground water as well as solution channels and open fractures.

2 Distribution phenomena

a Dispersion of colloidal, soluble and suspended substances

b Diffusion of temperatures or gaseous substances

III TYPES OF STUDIES

Tracers are used in many situations, including:

A Treatment Plant Units

B Closed Conduits

C Open Channels

D Large Water Bodies

E Hydraulic Models

F Subsurface Aquifers

G Subsurface Basins

IV TYPES OF TRACERS

Materials used for tracers include:

A Floats

1 Surface (wooden and plastic devices as well as fruit). Influenced by wind action and debris.

2 Sub-surface "drogues". Apparent direction must be carefully evaluated.

B Salts

1 Common salt - hard to detect when less than 1 mg/l.

2 Brackish and freshwater mixing in estuaries and coastal aquifers.

3 Ammonium chloride. (See Table 1)

C Dyes, such as:

1 Rhodamine series - See section VI

2 Pontacyl Brilliant Pink B - most stable of fluorescent dyes. Rather expensive dye.

3 Fluorescein - very inexpensive. Fluoresces very near natural stream background level.

4 Uranine - fluoresces near stream background level.

D Radioactive substances, such as: (see Table 2) AEC must approve all experiments. AEC has published tables on permissible concentrations in unrestricted areas.(13)

1 Rubidium-86

2 Iodine-131

3 Tritium (H^3) gives best overall performance in subsurface(6) tracing.

4 Krypton-85 - used recently in gas transfer measurement in laboratory and stream studies.(12)

E Waste return characteristics; significant built-in factors such as:

1 Silt - understandably dependent upon velocity and obstructions.

2 Foam - ABS will foam at levels as low as 1 - 5 mg/l depending on hardness.

Table 1. SUBSURFACE NONRADIOISOTOPE FLUID TRACERS

Tracer	Form	Test conditions	Remarks
Chloride Dextrose Fluorescein Dye	NaCl Sugar	Lab: Columns of sand and sandy loam.	Chloride most rapid. Fluorescein far from satisfactory.
Chloride Dextrose Fluorescein Dye	NaCl Sugar	Field: 4 feet thick aquifer of sand and gravel, 90 feet below surface.	Fluorescein considered best of dyes. Chloride in the large conc. required caused density currents.
Dextrose Ammonia Fluorescein Dye Boron	Sugar Chloride H_3BO_3	Field: Added in Conc. of 10,000 ppm, 500 ppm, 50 ppm, and 200 ppm respectively into a 4 foot thick sand horizon at 2,100 feet below surface in McKean County, Pennsylvania.	Adsorption observed: dextrose - little or none, boron - slight (conc. dropped 30%) ammonia - moderate, fluorescein - strong (moved at rate about 1/10 that of flood water). Greenburg(33) found that borax in Hanford fine sandy loam penetrated only 2 feet.
Boron	$Na_2B_4O_7 \cdot 10H_2O$ H_3BO_3	Field: Injection into input wells. Dilution factor ~5.	Limit of detection is reached after 10^3 dilution.
Thiocyanate	NH_4SCN Alkali-Metal SCN	Field: Water flooding.	Dilution possible 10^5.
Rhodamine B		Field: Karst topography.	Dye not detected after 6×10^6 dilution.
Oxygen-18 Deuterium	H_2O HDO		Variations resulting from seasonal fluctuations are useful to study climate. (Also useful in meteorology and glaciology).

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Table 2. SUBSURFACE RADIOISOTOPE FLUID TRACERS - FIELD TESTS(6)

Tracer	Half-life	Form	Test conditions	Remarks
H^3	12.5 y.	HTO	Karst groundwater	Useful results up to 30 km distance.
			Chalk River soil, undisturbed, but penetrated with driven piezometers.	Fluorescein dye traveled only about 3/4 as fast as tritium.
			Glaciofluvial sand and gravel, various till soils, and fissures and channels.	Chromium complex traveled as fast as tritium.
S^{35}	89. d	Na_2SO_4	Chalk River soil at waste disposal site.	Lags behind tritium indicating some interaction with the soil. In soil containing appreciable amounts of gypsum there would be exchange and loss of S^{35}.
I^{131}	8 d.	Iodine, carrier free	4 feet thick aquifer of sand and gravel, 90 feet below surface.	Should be used with several ppm stable iodine carrier.
I^{131} Co^{60}	8 d. 5.2 y.	KI EDTA - (Co) and $K_3Co(CN)_6$	Tracer compounds used with carriers KI, EDTA-(Na) and $K_3Co(CN)_6$. Limestone aquifer consisted of some argillaceous material and marly dolomite. Single-well pulse technique.	Co^{60} complex unsuitable in this aquifer. $K_3Co^{60}(CN)$ appeared to be most suitable of tracers tested in limestone aquifer.
Br^{82}	36 h.	NH_4Br	Aquifers in alluvium and permeable strata in calcareous marl series.	Br^{82} satisfactory under these conditions.
Rb^{86}	19 d.	RbCl	Groundwater direction at reservoir site in Egypt.	Tracer detectable 7 miles from injection after 5 d.
I^{131} P^{32}	8 d. 14 d.	NaI Na_2HPO_4	Single pulse tracing at dam site for Jesenitz Reservoir on Odrava River in Czechoslovakia.	Detected very high subterranean water velocity necessitating sealing of porous layer.
I^{131} Br^{82} Na^{24}	8 d. 36 h. 15 h.	NaI NaBr NaCl	Single pulse tracing at dam site on arges River in Roumania.	Determined direction and velocity of flow of subsurface water.

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3 Acid - severe effects upon total stream ecology.

4 Temperature - infra-red filming techniques reveal 1°C temperature differences.

5 Lignins - "Orzan" is a commercial product based upon these non-degrading materials.

6 Dyes - prevalent in textile wastes.

F Biota, various techniques possible with:

1 Mammals

2 Fish

3 Shellfish

V DESIRABLE CHARACTERISTICS

Ideally a tracer should be:

A Biologically innocuous to human and aquatic life, with special reference to

1 Acute toxicity

2 Long-term toxicity

3 Carcinogenic effects

4 Genetic effects

B Stable or persistant despite the effects of

1 Stream chemical constituents

2 Bacteria

3 Sunlight

4 Adsorption

5 Temperature

6 Wind action

7 Inherent decay

8 Channel obstructions

9 Stratification

C Readily detectable either

1 Visually and/or

2 Instrumentally despite dilution or background effects;

D Representative: coincide with the real waste and stream blend under study: this involves miscibility and specific gravity characteristics similar to the blend.

E Economic: this involves careful evaluation of the costs for

1 Materials

2 Material preparation, and release - ease of handling

3 Detection equipment

4 Detection technique and recording - convenience in operation

5 Stream deterioration

F Esthetically agreeable: inoffensive to:

1 Taste

2 Sight - conflict with easy detection

VI ELEMENTS OF A TRACER STUDY - USING RHODAMINE DYES (Currently in widespread use for surface studies.)

A Rhodamine Dyes

1 Available from American Cyanamid, DuPont, Allied Chemical and General Aniline.

2 Price $4.95 per pound except Rhodamine WT which is $10.00 per pound.

3 Specific gravity of 0.99 to 1.12 depending on the proportions of Alcohol, Ethylene Glycol, Acetic Acid, and Water used as solvents in preparing the solution.

4 Solutions normally 15%, 20%, 30%, or 40% dye by weight.

5 pH 1.0 to 9.0.

6 Peak wavelength of adsorption at 525-556μ and of fluorescence at 548-585μ are not normally found in natural waters.

7 Detectable to 0.5 μg/l with continuously sampling and recording equipment.

8 Visibly red to approximately 1.0 mg/l.

9 Rapid dispersion when dropped in water.

10 Subject to some destruction by light, but much less than experienced with fluorescein.

11 Destroyed by agents such as hypochlorite. Rhodamine WT is more resistant.

12 Essentially non-toxic.

B Measuring Equipment

1 Available from Turner Associates, Beckman Instruments, Inc., American Instrument Co., Inc.

2 Price as low as $1,000 for photofluorometer complete or $500 for unit to modify a spectrophotometer.

3 Available for individual grab sample analysis or continuous analysis of sample pumped through instrument.

4 Automatic recorders available.

5 Will measure several dyes (fluorescein, rhodamine, etc.)

C Fluorometer Calibration

The instrument should be calibrated before and after a study. The calibration is a function of temperature (about 2.3%/°C) so two or more runs with temperatures covering the expected range of the study water are desirable. Also it is advisable to make a calibration with the study water. This will check for background and any difference in fluorescent characteristics. A few helpful hints are:

1 Pumping a known concentration sample, from a plastic bag immersed in a constant temperature water bath, through the meter is a convenient calibration procedure. Continuous dousing with a concentrated solution into the bag will give various instrument readings through the detectable ranges.

2 Flush the system with methyl or butyl alcohol solution before each calibration to remove traces of dye.

3 Bubbles in the flow cuvette will cause erratic readings.

4 The fluorometer door is not light tight, so the instrument should be draped and used out of intense light to prevent erroneous high readings.

5 The sample lines on a continuous flow system will transmit light through the instrument door causing erroneous high readings. Taping these tubes with black electrical tape for about one foot will alleviate problem.

D Study Procedures

1 Select objectives which then dictate:

a Length of time to be studied

b Release point

c Monitoring locations and schedules

2 Determine physical properties of affected area.

a Probable net flows

b Total water volumes

c Probable water temperature

d Natural fluorescent background

e Salinity

f Suspended solids

3 Estimate required quantities of dye

4 Calibrate fluorometers

5 Conduct mock run to test equipment

6 Public relations

7 Release and monitor dye

8 Process and interpret data

9 Report

VIII DISPERSION STUDY COMPUTATIONS

A Most Dispersion Equations are Normalized Solutions of Ficks Second Law

$$\frac{\partial c}{\partial t} = D_x \frac{\partial^2 c}{\partial x^2}$$

c = concentration, x = distance,
t = time, D_x = diffusion coefficient

B Hydraulic Model Studies of the Fate of Pollution

From instantaneous release compute steady state nonconservative pollutant distribution

$$C_{(xy)} = \int f(t) + g(t)$$

where $f(t)$ represents concentration function and $g(t)$ = decay function

C Coastal Water Dispersion Study, Hilo Bay, Hawaii(9)

1 From instantaneous release measure concentration profile during slack water

2 Using these measurements and a two dimensional dispersion model compute D_x and D_y.

3 Two dimensional model

$$\frac{\partial c}{\partial t} = D_x \frac{\partial^2 c}{\partial x^2} + D_y \frac{\partial^2 c}{\partial y^2} - \bar{U}\frac{\partial c}{\partial x} - \bar{V}\frac{\partial c}{\partial y}$$

$\bar{U}$ = velocity in x direction
$\bar{V}$ = velocity in y direction

Normalized solution:

$$C = \frac{W_D}{2\pi\sigma_x\sigma_y} \exp - \frac{1}{2}\left[\left(x - \frac{ut}{\sigma_x}\right)^2 + \left(y - \frac{vt}{\sigma_y}\right)^2\right]$$

D Channel Studies

1 From instantaneous release compute longitudinal dispersion coefficient

2 Measure time-concentration profile at given station

3 This C vs. t curve fits equation

$$C = \frac{M}{A(4\pi Dt)^{\frac{1}{2}}} \exp - \frac{(x - ut)^2}{4\,Dt}$$

4 Take log of both sides

$$Ct^{\frac{1}{2}} = \log\left(\frac{M}{A\,4\pi D}\right) - \frac{(x - ut)^2}{4\,Dt}\log e$$

5 D can be determined from semi-log

plot of $Ct^{\frac{1}{2}}$ vs. $\frac{(x - ut)^2}{t}$

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Descriptors: Fluorescence, Fluorescent Dye, Path of Pollutants, Radioactivity Techniques, Radioisotopes, Tracers

SAMPLING IN WATER QUALITY STUDIES

I INTRODUCTION

A Objective of Sampling

1 Water quality characteristics are not uniform from one body of water to another, from place to place in a given body of water, or even from time to time at a fixed location in a given body of water. A sampling program should recognize such variations and provide a basis for interpretation of their effects.

2 The purpose of collection of samples is the accumulation of data which can be used to interpret the quality or condition of the water under investigation. Ideally, the sampling program should be so designed that a statistical confidence limit may be associated with each element of data.

3 Water quality surveys are undertaken for a great variety of reasons. The overall objectives of each survey greatly influence the location of sampling stations, sample type, scheduling of sample collections, and other factors. This influence should always be kept in mind during planning of the survey.

4 The sampling and testing program should be established in accordance with principles which will permit valid interpretation.

a The collection, handling, and testing of each sample should be scheduled and conducted in such a manner as to assure that the results will be truly representative of the sources of the individual samples at the time and place of collection;

b The locations of sampling stations and the schedule of sample collections for the total sampling program should be established in such a manner that the stated investigational objectives will be met; and

c Sampling should be sufficiently repetitive over a period of time to provide valid data about the condition or quality of the water.

B Sample Variations

Interpretation of survey data is based on recognition that variations will occur in results from individual samples. While it is beyond the scope of this discussion to consider the implications of each in detail, the following can be identified as factors producing variations in data and should be considered in planning the sampling program.

1 Apparent Variations

a Variations of a statistical nature, due to collection of samples from the whole body of water, as contrasted with examination of all the water in the system.

b Variations due to inherent precision of the analytical procedures.

c Apparent variations are usually amenable to statistical analysis.

2 True Differences

a Variations of a cyclic nature

Diurnal variations, related to alternating periods of sunlight and darkness.

Dirunal variations related to waste discharges from communities.

Seasonal variations, related to temperature and its subsequent effects on chemical and biological processes and interrelationships.

Variations due to tidal influences, in coastal and estuarine waters.

b Intermittent variations

Dilution by rainfall and runoff.

Effects of irregular or intermittent discharges of wastewater, such as "slugs" of industrial wastes.

Irregular release of water from impoundments, as from power plants.

c Continuing changes in water quality

Effects downstream from points of continuous release of wastewater.

Effects of confluence with other bodies of water.

Effects of passage of the water through or over geological formations of such chemical or physical nature as to alter the characteristics of the water.

Continuing interactions of biological, physical, and chemical factors in the water, such as in the process of natural self-purification following introduction of organic contaminants in a body of water.

II LOCATION OF SAMPLING STATIONS

A The Influence of Survey Objectives

Much of the sampling design will be governed by the stated purpose of the water investigation. As an example of how different objectives might influence sampling design, consider a watercourse with points A and B located as indicated in Figure 1.

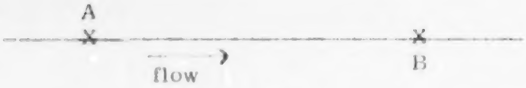


Figure 1

Point A can be the point of discharge of wastes from Community A. Point B can be any of several things, such as an intake of water treatment plant supplying Community B, or it might be the place where the river crosses a political boundary, or it may be the place where the water is subject to some legitimate use, such as for fisheries or for recreational use.

1 Assume that the objective of a water quality investigation is to determine whether designated standards of water quality are met at a water plant intake at Point B. In this case, the objective only is concerned with the quality of the water as it is available at Point B. Sampling will be conducted only at Point B.

2 Alternately, consider that there is a recognized unsatisfactory water quality at Point B, and it is alleged that this is due to discharges of inadequately treated wastes, originating at Point A. Assume that the charge is to investigate this allegation.

In this case the selected sampling sites will include at least three elements:

a At least one sampling site will be located upstream from Point A, to establish base levels of water quality, and to check the possibility that the observed conditions actually originated at some point upstream from Point A.

b A site or sites must be located downstream from Point A. Such a site should be downstream a sufficient distance to permit adequate mixing in the receiving water.

c Sampling would be necessary at Point B in order to demonstrate that the water quality is in fact impaired, and that the impairment is due to influences traced from Point A.

B Hydraulic Factors

1 Flow rate and direction

a In a survey of an extended body of water it is necessary to determine the rate and direction of water movement influences selection of sampling sites. Many workers plan sampling stations representing not less than the distance water flows in a 24-hour period. Thus, in Figure 1, intervening sampling stations would be selected at points representing the distance water would flow in about 24 hours.

b In a lake or impoundment direction of flow is the major problem influencing selection of sampling stations. Frequently it is necessary to establish some sort of grid network of stations in the vicinity of the suspected sources of pollution.

c In a tidal estuary, the oscillating nature of water movement will require establishment of sampling stations in both directions from suspected sources of pollution.

2 Introduction of other water

a In situations in which a stream being studied is joined by another stream of significant size and character, sampling stations will be located immediately above the extraneous stream, in the extraneous stream above its point of juncture with the main stream, and in the main stream below the point of juncture.

b Similar stations will be needed with respect to other water discharges, such as from industrial outfalls, other communities, or other installations in which water is introduced into the main stream.

3 Mixing

a Wherever possible, one sampling point at a sample collection site is used in stream surveys. This usually is near the surface of the water, in the main channel of flow.

b In some streams mixing does not occur quickly, and introduced water moves downstream for considerable distances below the point of confluence with the main streams. Example: Susquehanna River at Harrisburg, where 3 such streams are recognizable in the main river. Preliminary survey operations should identify such situations.

When necessary, collect separate samples at two or more points across the body of water.

c Similarly, vertical mixing may not be rapid. This is noted particularly in tidal estuaries, where it may be necessary to make collections both from near the bottom and near the surface of the water.

d Collection of multiple samples from a station requires close coordination with the laboratory, in terms of the number of samples that can be examined. Some types of samples may be composited. The decision must be reached separately for each type of sample.

C Types of Analytical Procedure

1 Samples collected for physical, chemical, and bacteriological tests and measurements may be collected from the same series of sampling stations.

2 Sampling stations selected for biological (ecological) investigation require selection of a series of similar aquatic habitats (a series of riffle areas, or a series of pool areas, or both). The sites used by the aquatic biologist may or may not be compatible with those used for the rest of the survey. Accordingly, in a given stream survey, the stations used by the aquatic biologist usually are somewhat different from the stations used for other examinations.

D Access to Sampling Stations

For practical reasons, the sampling site should be easily reached by automobile if a stream survey, or by boat if the survey is on a large body of water. Highway bridges are particularly useful, if the sample collector can operate in safety.

III FACTORS IN SCHEDULING OF SAMPLING PROGRAMS

A Survey Objectives

B Time of Year

1 In short-term water quality investigations, particularly in pollution investigations, there often is need to demonstrate the extremes of pollution effects on the aquatic environment. For this reason, many short-term surveys are conducted during the warmer season of the year, at such times as the water flow rate and volume is at a minimum and there is minimum likelihood of extensive rainfall.

2 In a long-term investigation, sampling typically is conducted at all seasons of the year.

C Daily Schedules

As shown in an introductory paragraph, water quality is subject to numerous cyclic or intermittent variations. Scheduling of sample collections should be designed to reveal such variations.

1 In short-term surveys it is common practice to collect samples from each sampling site at stated intervals through the 24-hour day, continuing the program for 1 - 3 weeks. Sampling at 3-hour intervals is preferred by many workers, though practical considerations may require extension to 4- or even 6-hour intervals.

2 In an extended survey there is a tendency to collect samples from each site at not more than daily intervals, or even longer. In such cases the hour of the day should be varied through the entire program, in order that the final survey show cyclic or intermittent variations if they exist.

3 In addition, sampling in tidal waters requires consideration of tidal flows. If samples are collected but once daily, many workers prefer to make the collections at low slack tide.

4 In long-term or any other survey in which only once-daily samples are collected, it is desirable to have an occasional period of around-the-clock sampling.

IV IDENTIFICATION OF SAMPLING SITES

A River Mile System

The FWPCA method of identifying points on a water course is by counting river miles from the mouth (or junction with a larger stream) back to the source. This should not be confused with other systems, such as those in which the river mile is started at the source of the stream and proceeds to the mouth of the stream or confluence with another body of water.

B STORET System

The STORET System is a computer-oriented data processing system used by FWPCA for storage, retrieval, and analysis of water quality data collected by federal, state, local, and private agencies.

The system includes a complex system - based on the river mile system - for identifying sampling locations on all rivers and streams in the United States. A recent addition to the system introduces a location procedure based on geographic coordinates; this procedure is especially adapted to location of sampling stations in large bodies of water such as lakes and impoundments.

Not all locations have been coded at this time, although the coding systems have been established. The interested worker should consult Public Health Service Publication No. 1263, "The Storage and Retrieval of Data for Water Quality Control." 1963.

V SAMPLE COLLECTION

A Types of Samples

1 "Grab" sample - a grab sample is usually a manually collected single portion of the wastewater or stream water. An analysis of a grab sample shows the concentration of the constituents in the water at the time the sample was taken.

2 "Continuous" sample - when several points are to be sampled at frequent intervals or when a continuous record of quality at a given sampling station is required, an automatic or continuous sampler may be employed.

a Some automatic samplers collect a given volume of sample at definite time intervals; this is satisfactory when the volume of flow is constant.

b Other automatic samplers take samples at variable rates in proportion to changing rates of flow. This type of sampler requires some type of flow measuring device.

3 "Composite" sample - a composite sample is the collection and mixing together of various individual samples based upon the ratio of the volume of flow at the time the individual samples were taken to the total cumulative volume of flow. The desired composite period will dictate the magnitude of the cumulative volume of flow. The more frequently the samples are collected, the more representative will be the composite sample to the actual situation. Composite samples may be obtained by:

a Manual sampling and volume of flow determination made when each sample is taken.

b Constant automatic sampling (equal volumes of sample taken each time) with flow determinations made as each sample is taken.

c Automatic sampling which takes samples at pre-determined time intervals and the volume of sample taken is proportional to the volume of flow at any given time.

B Type of Sampling Equipment

1 Manual sampling

a Equipment is specially designed for collection of samples from the bottom muds, at various depths, or at water surfaces. Special designs are related to protection of sample integrity in terms of the water characteristic or component being measured.

b For details of typical sampling equipment used in water quality surveys, see outlines dealing with biological, bacteriological, and chemical tests in this manual.

c Manual sampling equipment has very broad application in field work, as great mobility of operation is possible, at lower cost than may be possible with automatic sampling equipment.

2 Automatic sampling equipment

Automatic sampling equipment has several important advantages over manual methods. Probably the most important consideration is the reduction in personnel requirements resulting from the use of this equipment. It also allows more frequent sampling than is practical manually, and eliminates many of the human errors inherent in manual sampling.

Automatic sampling equipment has some disadvantages. Probably the most important of these is the tendency of many automatic devices to become clogged when liquids high in solids are being sampled. Individual portions of composite samples are usually quite small which may in some cases be disadvantageous. In using automatic samplers, sampling points are fixed, which results in a certain loss of mobility as compared to manual methods.

Automatic sampling equipment should not be used indiscriminately; some types of samples - notably bacteriological, biological, and DO samples - should not be composited. In cases of doubt, the appropriate analyst should be consulted.

a Compositing samplers

1) Jar and tube sampler - this type samples effectively when flow is nearly constant. As water drains from the upper carboy, the vacuum created syphons waste into the lower one. The rate-of-flow is regulated by the pinch clamp to fill the lower carboy during the sampling period. (See Figure 2)

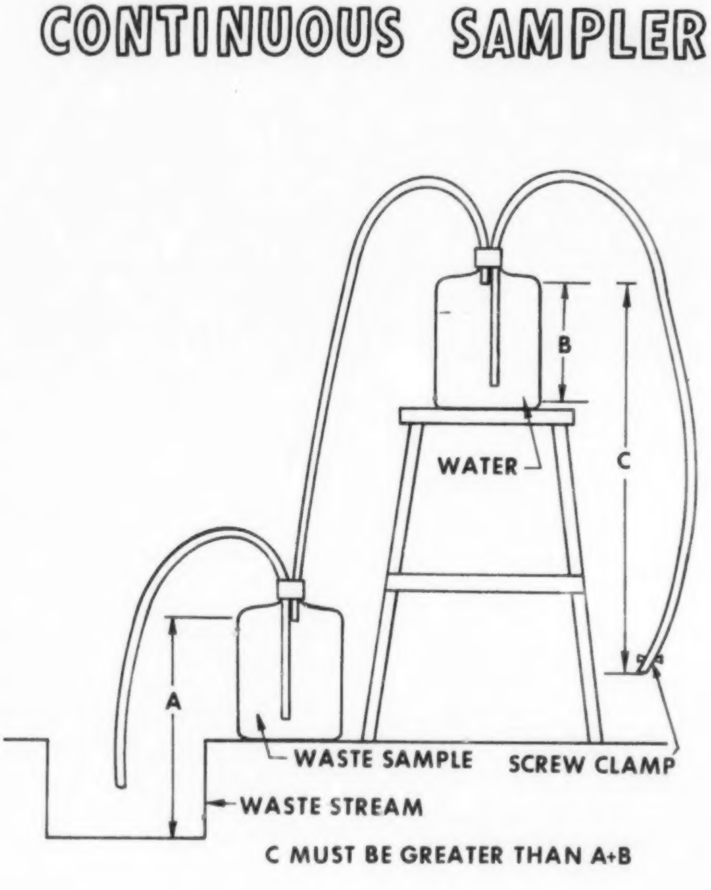


Figure 2

2) Scoop type

a) Rotating scoop

This device consists of a power driven scoop mounted upstream from a weir. The scoop is so designed and mounted that the sample volume grabbed on each rotation of the scoop is proportional to the flow, as governed by the head on the weir. The scoop may be rotated at a constant speed or timed to sample at fixed time intervals.

b) Revolving wheel with cups (Figure 3)

This device consists of a power driven wheel or disc mounted upstream from a weir. A number of freely suspended buckets are mounted at varying distances from the axis so that increased flow will cause more buckets to be filled, thereby giving a sample proportionate to flow. Both this device and the rotating scoop sampler can, of course, be used for non-proportionate sampling.

c) Bucket elevators

This device may consist of a single bucket alternately lowered into and raised out of the waste stream, or it may consist of a series of buckets on an endless chain passing through the waste stream. In either case, it will include a tripping mechanism to cause the bucket or buckets to spill into a sampling funnel. Both types may be operated continuously or timed for intermittent operation. This method is not well adapted to proportionate sampling.

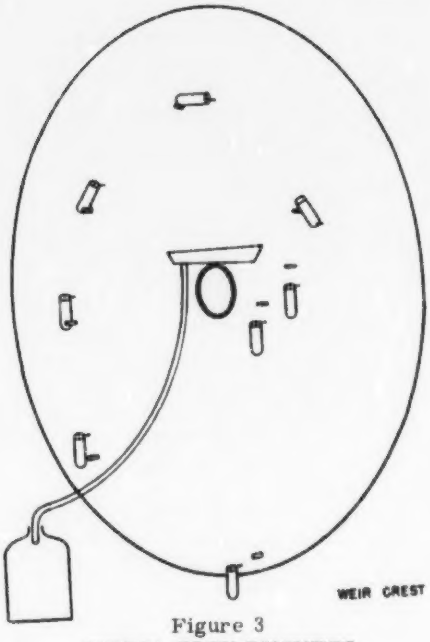


Figure 3
WHEEL WITH BUCKETS

3 Pumps

a) Chemical feed pumps have been found useful for sampling, because of their ability to meter out small doses of liquids. A timing mechanism may be used to make the pump run for longer periods during heavy flow, thereby allowing collection of the sample in proportion to flow. These pumps are usually provided with adjustable stroke and variable speed features which allow variation of the volume of sample being pumped. Figure 4 illustrates a battery operated pump.

b) Automatic shift sampler (Figure 5)

Figure 5 shows the automatic shift sampler. It consists first of a Randolph or other "squeegee-" type pump unit.

The 2-rpm gear motor drives the pump at between 1 and 2 rpm through the spring-loaded adjustable-pitch pulley and adjustable motor-base arrangement.

We use 1/8-in. (.32-cm) ID or 1/4-in. (.64-cm) ID polyethylene tubing for sample intake from the waste stream. The sample flow is delivered to the distributor via a 3/16-in. (.48-cm) ID Tygon tube which is supported loosely by a wire attached to the framework.

Operation of the distributor is very simple. The 1-rpm clock motor powers the chain-and-sprocket drive which turns a threaded bolt. Rotation of the bolt moves the discharge tube down the plastic trough at a rate equal to one division every eight hours. With the 10 sample-jar receivers the timer can be set on Friday, and the 9 week-end shift samples can be picked up on Monday.

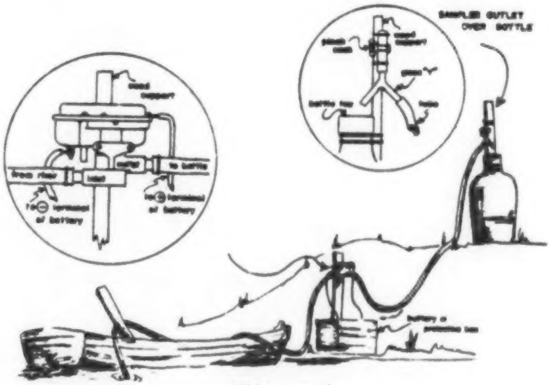
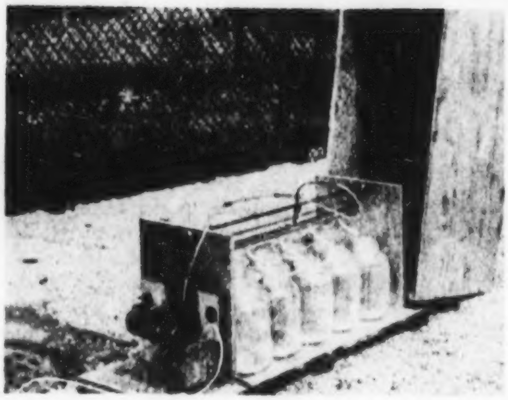

SAMPLER OUTLET OVER BOTTLE
wood support
from river
inlet
outlet
to bottle
to ⊖ terminal of battery
to ⊕ terminal of battery
battery in protective box

Figure 4
BATTERY OPERATED PUMP



4) Solenoid-valve arrangements

A solenoid valve employed in connection with a timing device may be used for withdrawing waste from a pipe under pressure. Used in connection with a pump such devices may be employed in sampling free flowing streams. (See Figures 6 and 7.)

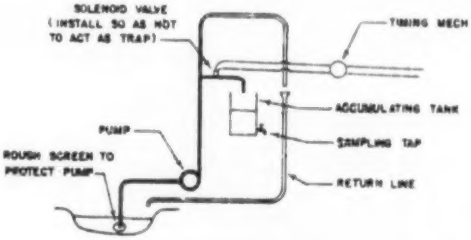

SOLENOID VALVE (INSTALL SO AS NOT TO ACT AS TRAP)
TIMING MECH
ACCUMULATING TANK
SAMPLING TAP
PUMP
ROUGH SCREEN TO PROTECT PUMP
RETURN LINE

Figure 6
PUMP - SOLENOID VALVE - TIMER TYPE SAMPLER

5) Vacuum operated

In its simplest form, the vacuum is created by a suitably mounted siphon. It collects the sample at a uniform rate and is not suitable for use when proportional sampling is required.

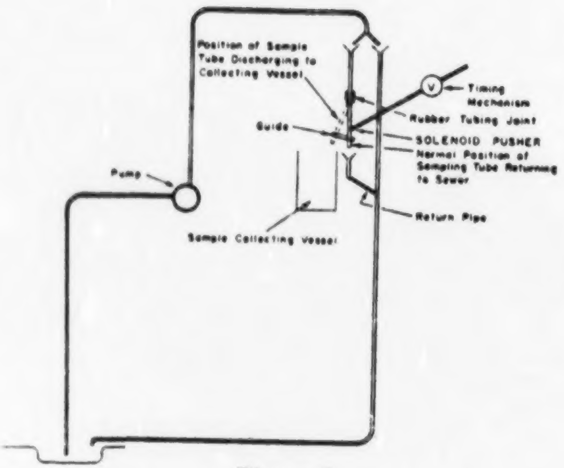

Position of Sample
Tube Discharging to
Collecting Vessel
Timing
Mechanism
Rubber Tubing Joint
Guide
SOLENOID PUSHER
Normal Position of
Sampling Tube Returning
to Sewer
Pump
Return Pipe
Sample Collecting Vessel

Figure 7
PUMP - SOLENOID-DIVERTER - TIMER
TYPE SAMPLER

6) Air vent control

This type of device is illustrated in Figure 8. The rate of sample collection is controlled by the bubbling mechanism. It is not suitable for use when proportionate sampling is required.

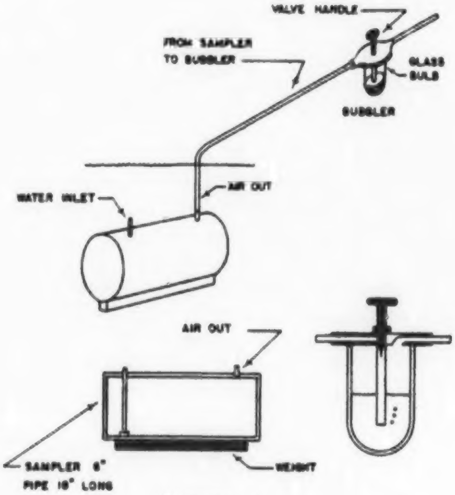

VALVE HANDLE
FROM SAMPLER
TO BUBBLER
GLASS
BULB
BUBBLER
WATER INLET
AIR OUT
AIR OUT
SAMPLER 6"
PIPE 18" LONG
WEIGHT

Figure 8
AIR RELEASE TYPE SAMPLER

7) Drip sampler

Two types of this device are illustrated in Figures 9 and 10. Both devices are simple methods of obtaining a composite sample at a fairly constant rate.

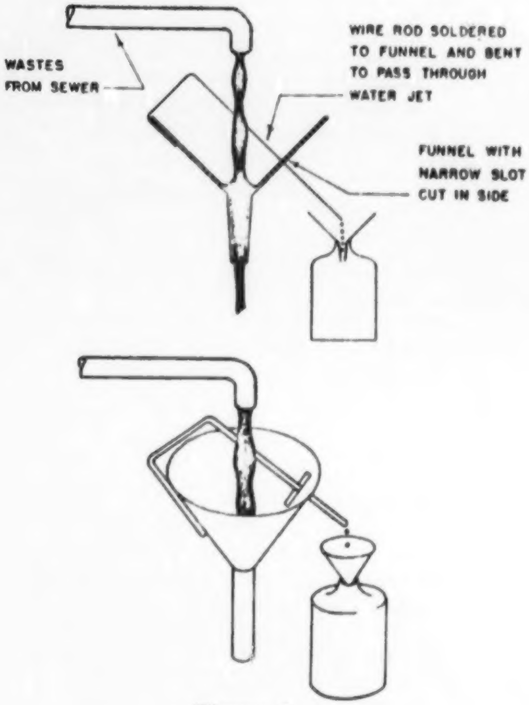

WASTES
FROM SEWER
WIRE ROD SOLDERED
TO FUNNEL AND BENT
TO PASS THROUGH
WATER JET
FUNNEL WITH
NARROW SLOT
CUT IN SIDE

Figure 9
FUNNEL AND ROD DIVERTER

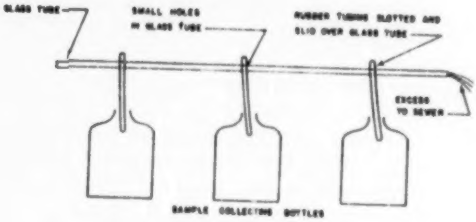

GLASS TUBE
SMALL HOLES
IN GLASS TUBE
RUBBER TUBING SLOTTED AND
SLID OVER GLASS TUBE
EXCESS
TO SEWER
SAMPLE COLLECTING BOTTLES

Figure 10
DRIP TUBE SAMPLERS

b Continuous recording equipment

Instruments have been developed which provide direct measurement of temperature, pH, conductivity, color, and dissolved oxygen. Such instruments may be equipped for continuous recording. Instruments of this type are quite expensive and their installation is often difficult. They are best adapted to permanent installations, although good portable non-recording instruments are available for the measurement of temperature, pH, and conductivity.

VI SOME CONSIDERATIONS IN SAMPLING OPERATIONS

All procedures in care and handling of samples between collection and the performance of observations and tests are directed toward maintaining the reliability of the sample as an indication of the characteristics of the sample source.

A Sample Quantity

1 Samples for a series of chemical analyses require determination of the total sample volume required for all the tests, and should include enough sample in addition to provide a safety factor for laboratory errors or accidents. Many workers collect about twice the amount of sample actually required for the chemical tests. As a rule of thumb, this is on the order or 2 liters.

2 Bacteriological samples, in general, are collected in 250 - 300 ml sterile bottles; approximately 150 - 200 ml of samples is adequate in practically all cases.

B Sample Identification

1 Sample identification must be maintained throughout any survey. It is vital, therefore, that adequate records be made of all information relative to the source of the sample and conditions under which the collection was made. All information must be clearly understandable and legible.

2 Every sample should be identified by means of a tag or bottle marking, firmly affixed to the sample bottle. Any written material should be with indelible marking material.

3 Minimum information on the sample label should include identification of the sample site, date and time of collection, and identification of the individual collecting the sample.

4 Supplemental identification of samples is strongly recommended, through maintenance of a sample collection logbook. If not included on the sample tag (some prefer to duplicate such information) the logbook can show not only the sample site and date and hour of collection, but also the results of any tests made on site (such as temperature, pH, dissolved oxygen). In addition, the logbook should provide for notation of any unusual observations made at the sampling site, such as rainfall, direction and strength of unusual winds, or evidence of disturbance of the collection site by human or other animal activity.

C Care and Handling of Samples

1 As a general policy, all observations and tests should be made as soon as possible after sample collection.

a Some measurements require performance at the sampling site, such as temperature, pH, dissolved oxygen, chlorine, flow rate, etc.

b Some tests are best made at the sampling site because the procedures are simple, rapid, and of acceptable accuracy. These may include such determinations as conductivity.

c Some additional determinations, such as alkalinity, hardness, and turbidity may be made in the field, provided that ease, convenience, and reliability of results are acceptable for the purposes of the study.

2 Samples to be analyzed in the laboratory require special protection to assure that the quality measured in the sample represents the condition of the source. Many samples, especially those subjected to biological analysis, require special preservation, protection, and handling procedures. In case of doubt, the appropriate analyst should be consulted. Most common procedures for sample protection include:

  a Examination within brief time after collection.

  b Temperature control.

  c Protection from light.

  d Addition of preservative chemicals.

  Applications of these sample protective procedures are along the following lines:

3 Early examination of sample

  Applicable to all types of samples.

4 Temperature control

  a All biological materials for examination in a living state should be iced between collection and examination.

  b Bacteriological samples should be iced during a maximum transport time of 6 hours. Such samples should be refrigerated upon receipt in the laboratory and processed within 2 hours.

  c Chemical samples often require icing

  Preservation by refrigeration at $4^{o}C$ is recommended for acidity, alkalinity, BOD, color, sulfate, threshold odor, and other samples. Holding times vary. Quick freezing will permit retention of many samples for up to several months prior to laboratory examination.

5 Protection from light

  a Any constituent of water which may be influenced by physiochemical reactions involving light should be protected. DO samples brought to the iodine stage, for example, should be protected from light prior to titration.

  b In addition, any water constituent (such as dissolved oxygen) which may be influenced by algal activity should be protected from light.

6 Addition of chemical preservatives

  a Bacteriological samples never should be "protected" by addition of preservative agents. The only permissible chemical additive is sodium thiosulfate, which is used to neutralize free residual chlorine, if present.

  b Samples for biological examination should be protected by chemical additives only under specific direction of the principal biologist in a water quality study.

  c For chemical tests, preservatives are useful for a number of water components. The following examples are cited:

  Nitrogen and phosphorus analyses:

  The addition of 40 mg $HgCl_2$ per liter of sample and refrigeration at $4^{o}C$ will retard biological activity which might otherwise alter the concentration of these constituents

Metals: The addition of 5 ml of HNO_3 acid per liter of sample prevents precipitation of the metal in the container.

COD and Organic Carbon: Addition of 2 ml sulfuric acid per liter of sample is suggested.

In general, samples requiring retardation of biological activity can be temporarily preserved by addition of chloroform; tests should be run as soon as possible, however.

Cyanide determinations may be delayed temporarily through addition of alkali to the sample. Addition of sodium hydroxide to pH 10 is recommended.

Sulfide analysis may be delayed up to 7 days by addition of 2 ml/liter of sample of 2N solution of zinc acetate.

Phenol analysis can be delayed temporarily by acidification to pH 4.0 with phosphoric acid and preservation with 1 gram $CuSO_4 \cdot 5H_2O$ per liter of sample.

REFERENCES

1 Standard Methods for the Examination Water, Sewage and Industrial Wastes. 13th Ed. A.P.H.A. 1971

2 Planning and Making Industrial Waste Surveys. Ohio River Valley Water Sanitation Commission.

3 Industry's Idea Clinic. Journal of the Water Pollution Control Federation. April, 1965.

This outline was prepared by H. L. Jeter, Director, National Training Center, EPA, WPO, Cincinnati, OH 45268 and P.F. Atkins, Jr., formerly Sanitary Engineer, Training Activities.

Descriptors: Instrumentation, Investigations, Natural Streams, On-Site Investigations, Preservation, Samplers, Sampling, Water Analysis, Water Sampling

SAMPLE HANDLING - FIELD THROUGH LABORATORY

I PLANNING A SAMPLING PROGRAM

A Factors to Consider:

1 Locating sampling sites

2 Sampling equipment

3 Type of sample required

a grab
b composite

4 Amount of sample required

5 Frequency of collection

6 Preservation measures, if any

B Decisive Criteria

1 Nature of the sample source

2 Stability of constituent(s) to be measured

3 Ultimate use of data

II REPRESENTATIVE SAMPLES

If a sample is to provide meaningful and valid data about the parent population, it must be representative of the conditions existing in that parent source at the sampling location.

A The container should be rinsed two or three times with the water to be collected.

B Compositing Samples

1 For some sources, a composite of samples is made which will represent the average situation for stable constituents.

2 The nature of the constituent to be determined may require a series of separate samples.

C The equipment used to collect the sample is an important factor to consider. ASTM(1) has a detailed section on various sampling devices and techniques.

D Great care must be exercised when collecting samples in sludge or mud areas and near benthic deposits. No definite procedure can be given, but careful effort should be made to obtain a representative sample.

III SAMPLE IDENTIFICATION

A Each sample must be unmistakably identified, preferably with a tag or label. The required information should be planned in advance.

B An information form preprinted on the tags or labels provides uniformity of sample records, assists the sampler, and helps ensure that vital information will not be omitted.

C Useful Identification Information includes:

1 sample identity code
2 signature of sampler
3 signature of witness
4 description of sampling location detailed enough to accommodate reproducible sampling. (It may be more convenient to record the details in the field record book).
5 sampling equipment used
6 date of collection
7 time of collection
8 type of sample (grab or composite)
9 water temperature
10 sampling conditions such as weather, water level, flow rate of source, etc.
11 any preservative additions or techniques
12 record of any determinations done in the field
13 type of analyses to be done in laboratory

IV SAMPLE CONTAINERS

A Available Materials

1 glass
2 plastic
3 hard rubber

B Considerations

1 Nature of the sample - Organics attack polyethylene.

2 Nature of constituent(s) to be determined - Cations can adsorb readily on some plastics and on certain glassware. Metal or aluminum foil cap liners can interfere with metal analyses.

3 Preservatives to be used - Mineral acids attack some plastics.

4 Mailing Requirements - Containers should be large enough to allow extra volume for effects of temperature changes during transit. All caps should be securely in place. Glass containers must be protected against breakage. Styrofoam linings are useful for protecting glassware.

C Preliminary Check

Any question of possible interferences related to the sample container should be resolved before the study begins. A preliminary check should be made using corresponding sample materials, containers, preservatives and analysis.

D Cleaning

If new containers are to be used, preliminary cleaning is usually not necessary.

If the sample containers have been used previously, they should be carefully cleaned before use.

There are several cleaning methods available. Choosing the best method involves careful consideration of the nature of the sample and of the constituent(s) to be determined.

1 Phosphate detergents should not be used to clean containers for phosphorus samples.

2 Traces of dichromate cleaning solution will interfere with metal analyses.

E Storage

Sample containers should be stored and transported in a manner to assure their readiness for use.

V SAMPLE PRESERVATION

Every effort should be made to achieve the shortest possible interval between sample collection and analyses. If there must be a delay and it is long enough to produce significant changes in the sample, preservation measures are required.

At best, however, preservation efforts can only retard changes that inevitably continue after the sample is removed from the parent population.

A Functions

Methods of preservation are relatively limited. The primary functions of those employed are:

1 to retard biological action
2 to retard precipitation or the hydrolysis of chemical compounds and complexes
3 to reduce volatility of constituents

B General Methods

1 pH control - This affects precipitation of metals, salt formation and can inhibit bacterial action.

2 Chemical Addition - The choice of chemical depends on the change to be controlled.

Mercuric chloride is commonly used as a bacterial inhibitor. Disposal of the mercury-containing samples is a problem and efforts to find a substitute for this toxicant are underway.

To dispose of solutions of inorganic mercury salts, a recommended procedure is to capture and retain the mercury salts as the sulfide at a high pH. Several firms have tentatively agreed to accept the mercury sulfide for re-processing after preliminary conditions are met.(4)

3 Refrigeration and Freezing - This is the best preservation technique available, but it is not applicable to all types of samples. It is not always a practical technique for field operations.

C Specific Methods

The EPA Methods Manual(2) includes a table summarizing the holding times and preservation techniques for several analytical procedures. This information also can be found in the standard references(1, 2, 3) as part of the discussion of the determination of interest.

VI METHODS OF ANALYSIS

Standard reference books of analytical procedures to determine the physical and chemical characteristics of various types of water samples are available.

A EPA Methods Manual

The Analytical Quality Control Laboratory, National Environmental Research Center - Cincinnati, Environmental Protection Agency, has published a manual of analytical procedures to be used in Water Programs Operations laboratories for the analysis of water and wastes. The title of this manual is "Methods for Chemical Analysis of Water and Wastes" (1971).

For some procedures, the analyst is referred to Standard Methods and/or to ASTM Standards.

B Standard Methods

The American Public Health Association, the American Water Works Association and the Water Pollution Control Federation prepare and publish a volume describing methods of water analysis. These include physical and chemical procedures. The title of this book is "Standard Methods for the Examination of Water and Wastewater", 13th edition, 1971.

C ASTM Standards

The American Society for Testing and Materials publishes an annual "book" of specifications and methods for testing materials. The "book" currently consists of 33 parts. The part applicable to water is a book titled, "Annual Book of ASTM Standards, Part 23, Water; Atmospheric Analysis".

D Other References

Current literature and other books of analytical procedures with related information are available to the analyst.

VII ORDER OF ANALYSES

The ideal situation is to perform all analyses shortly after sample collection. In the practical order, this is rarely possible. The allowable holding time for preserved samples is the basis for scheduling analyses.

A The allowable holding time for samples depends on the nature of the sample, the stability of the constituent(s) to be determined and the conditions of storage.

1 For some constituents and physical values, immediate determination is required, e.g. dissolved oxygen, pH.

2 Using preservation techniques, the holding times for other determinations range from 6 hours (BOD) to 7 days (COD). Metals may be held up to 6 months.(2)

3 The EPA Methods Manual(2) includes a table summarizing holding times and preservation techniques for several analytical procedures. This information can also be found in the standard

references(1, 2, 3) as part of the discussion of the determination of interest.

4 If dissolved concentrations are sought, filtration should be done in the field if at all possible. Otherwise, the sample is filtered as soon as it is received in the laboratory. A 0.45 micron membrane filter is recommended for reproducible filtration.

B The time interval between collection and analysis is important and should be recorded in the laboratory record book.

VIII RECORD KEEPING

The importance of maintaining a bound, legible record of pertinent information on samples cannot be over-emphasized.

A Field Operations

A bound notebook should be used. Sample information written in it would include:

1 Sample identification records (See Part III)

2 Any information requested by the analyst as significant

3 Details of sample preservation

4 A complete record of data on any determinations done in the field. (See B, next)

5 Shipping details and records

B Laboratory Operations

Samples should be logged in as soon as received and the analyses performed as soon as possible.

A bound notebook should be used. Preprinted data forms provide uniformity of records and help ensure that required information will be recorded. Such sheets should be permanently bound.

Items in the laboratory notebook would include:

1 sample identifying code
2 date and time of collection
3 date and time of analysis
4 the analytical method used
5 any deviations from the analytical method used
6 data obtained during analysis
7 quality control checks used
8 any information useful to those who interpret and use the data
9 signature of the analyst

IX SUMMARY

Valid data can be obtained only from a representative sample, unmistakably identified, carefully collected and stored. A skilled analyst, using reliable methods of analyses and performing the determinations within the prescribed time limits, can produce data for the sample. This data will be of value only if a written record exists to verify sample history from the field through the laboratory.

REFERENCES

1 ASTM Standards, Part 23, Water; Atmospheric Analysis.

2 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, 1971, Cincinnati, OH 45268.

3 Standard Methods for the Examination of Water and Wastewater, 13th edition, APHA-AWWA-WPCF, 1971.

4 Dean, R., Williams, R. and Wise, R., Disposal of Mercury Wastes from Water Laboratories, Environmental Science and Technology, October, 1971.

This outline was prepared by A. Donahue, Chemist, National Training Center, EPA, WPO, Cincinnati, OH 45268.

Descriptors: Data Collections, Investigations, On-Site Data Collections, On-Site Investigations, Planning, Preservation, Sampling, Surveys, Water Analysis, Water Sampling

COLLECTION AND HANDLING OF SAMPLES FOR BACTERIOLOGICAL EXAMINATION

I INTRODUCTION

The first step in the examination of a water supply for bacteriological examination is careful collection and handling of samples. Information from bacteriological tests is useful in evaluating water purification, bacteriological potability, waste disposal, and industrial supply. Topics covered include: representative site selection, frequency, number, size of samples, satisfactory sample bottles, techniques of sampling, labeling, and transport.

II SELECTION OF SAMPLING LOCATIONS

The basis for locating sampling points is collection of representative samples.

A Take samples for potability testing from the distribution system through taps. Choose representative points covering the entire system. The tap itself should be clean and connected directly into the system. Avoid leaky faucets because of the danger of washing in extraneous bacteria. Wells with pumps may be considered similar to distribution systems.

B Grab samples from streams are frequently collected for control data or application of regulatory requirements. A grab sample can be taken in the stream near the surface.

C For intensive stream studies on source and extent of pollution, representative samples are taken by considering site, method and time of sampling. The sampling sites may be a compromise between physical limitations of the laboratory, detection of pollution peaks, and frequency of sample collection in certain types of surveys. First, decide how many samples are needed to be processed in a day. Second, decide whether to measure cycles of immediate pollution or more average pollution.

Sites for measuring cyclic pollution are immediately below the pollution source. Sampling is frequent, for example, every three hours.

A site designed to measure more average conditions is far enough downstream for a complete mixing of pollution and water. Keep in mind that averaging does not remove all variation but only minimizes sharp fluctuations. Downstream sites sampling may not need to be so frequent.

Samples may be collected 1/4, 1/2 and 3/4 of the stream width at each site or other distances, depending on survey objectives. Often only one sample in the channel of the stream is collected. Samples are usually taken near the surface.

D Samples from lakes or reservoirs are frequently collected at the drawoff and usually about the same depth and may be collected over this entire surface.

E Collect samples of bathing beach water at locations and times where the most bathers swim.

III NUMBER, FREQUENCY AND SIZE OF SAMPLES

A For determining sampling frequency for drinking water, consult the USPHS Standards.

1 The total number, frequency, and site are established by agreement with either state of PHS authorities.

2 The minimum number depends upon the number of users. Figure 1 indicates that the smaller populations call for relatively more samples than larger ones. The numbers on the left of the graph refer to actual users and not the population shown by census.

3 In the event that coliform limits of the standard are exceeded, daily samples must be taken at the same site. Examinations should continue until two consecutive samples show coliform level is satisfactory. Such samples are to be considered as special samples and shall not be included in the total number of samples examined.

4 Sampling programs described above represent a minimum number which may be increased by reviewing authority.

B For stream investigations the type of study governs frequency of sampling.

C Collect swimming pool samples when use is heavy. The high chlorine level rapidly reduces the count when the pool is not in use. Residual chlorine tests are necessary to check neutralization of chlorine in the sample.

D Lake beaches may be sampled as required depending on the water uses.

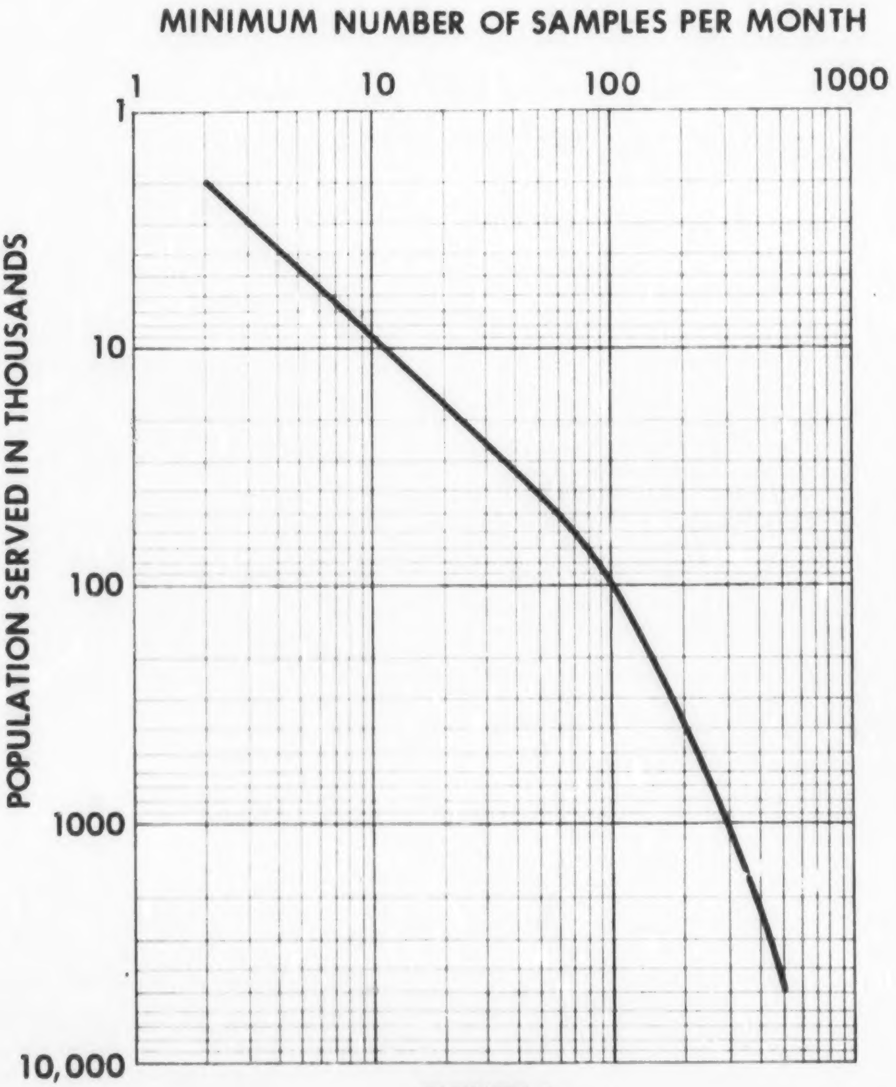


FIGURE 1

E Salt water or estuarine beaches are sampled as needed with frequency depending on use.

F Size of samples depends upon examination anticipated. Generally 100 ml is the minimum size.

IV BOTTLES FOR WATER SAMPLES

A The sample bottles should have capacity for at least 100 ml of sample, plus an air space. The bottle and cap must be of bacteriological inert materials. Resistant glass or heat resistant plastic are acceptable. At the National Training Center, wide mouth ground-glass stoppered bottles (Figure 2) are used.

All bottles must be properly washed and sterilized. Protect the top of the bottles and cap from contamination by paper or metal foil hoods. Both glass and heat resistant plastic bottles may be sterilized in an autoclave. Hold plastic at 121°C for at least 10 minutes. Hot air sterilization, 1 hour at 170°C, may be used for glass bottles.

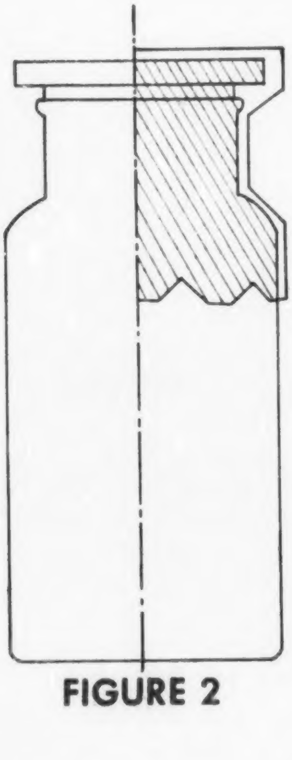


FIGURE 2

B Add sodium thiosulfate to bottles intended for halogenated water samples. A quantity of 0.1 ml of a 10% solution provides 100 mg per liter concentration in a 100 ml sample. This level shows no effect upon viability or growth.

C Supply catalogs list wide mouth ground glass stoppered bottles of borosilicate resistance glass, specially for water samples.

V TECHNIQUE OF SAMPLE COLLECTION

Follow aseptic technique as nearly as possible. Nothing but sample water must touch the inside of the bottle or cap. To avoid loss of sodium thiosulfate, fill the bottle directly and do not rinse. Always remember to leave an air space.

A In sampling from a distribution system, first run the faucet wide open until the service line is cleared. A time of 3-5 minutes generally is sufficient. Reduce the flow and fill the sample bottle without splashing. Some authorities stress flaming the tap before collection. A chlorine determination is often made on the site.

B The bottle may be dipped into some waters by hand. Avoid introduction of bacteria from the human hand and from surface debris. Some suggestions follow:
Hold the bottle near the base with one hand and with the other remove the hood and cap. Push the bottle rapidly into the water mouth down and tilt up towards the current to fill. A depth of about 6 inches is satisfactory. When there is no current move the bottle through the water horizontally and away from the hand. Lift the bottle from the water, spill a small amount of sample to provide an air space, and return the uncontaminated cap.

C Samples may be dipped from swimming pools. Determine residual chlorine on the pool water at the site. Test the sample at the laboratory to check chlorine neutralization by the thiosulfate.

D Sample bathing beach water by wading out to the two foot depth and dipping the sample up from about 6 inches below the surface. Use the procedure described in V. B.

E Wells with pumps are similar to distribution systems. With a hand pumped well, waste water for about five minutes before taking the sample. Sample a well without a pump by lowering a sterile bottle attached to a weight. A device which opens the bottle underneath the water will avoid contamination by surface debris.

F Various types of sampling devices are available where the sample point is inaccessible or depth samples are desired. The general problem is to put a sample bottle in place, open it, close it, and return it to the surface. No bacteria but those in the sample must enter the bottle.

1 The J - Z sampler described by Zobell in 1941, was designed for deep sea sampling but is useful elsewhere (Figure 3). It has a metal frame, breaking device for a glass tube, and sample bottle. The heavy metal messenger strikes the lever arm which breaks the glass tubing at a file mark. A bent rubber tube straightens and the water is drawn in several inches from the apparatus. Either glass or collapsible rubber bottles are sample containers.

Commercial adaptations are available.

2 Note the vane and lever mechanism on the New York State Conservation Department's sampler in Figure 4. When the apparatus is at proper depth the suspending line is given a sharp pull. Water inertia against the vane raises the stopper and water pours into the bottle. Sufficient sample is collected prior to the detachment of the stopper from the vane arm allowing a closure of the sample bottle.

The New York State Conservation Department's sampler is useful for shallow depths and requires nothing besides glass stoppered sample bottles.

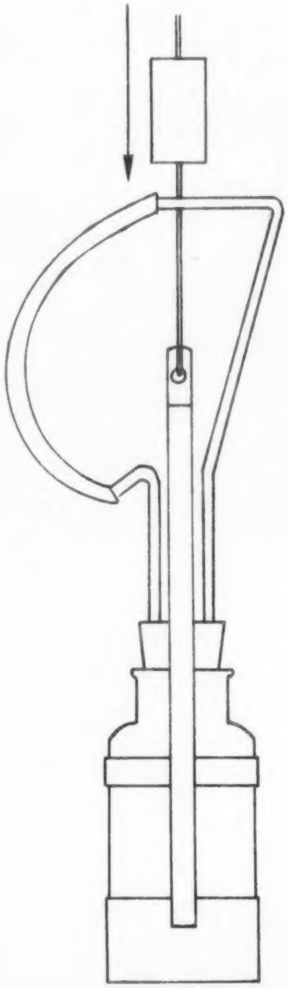


FIGURE 3

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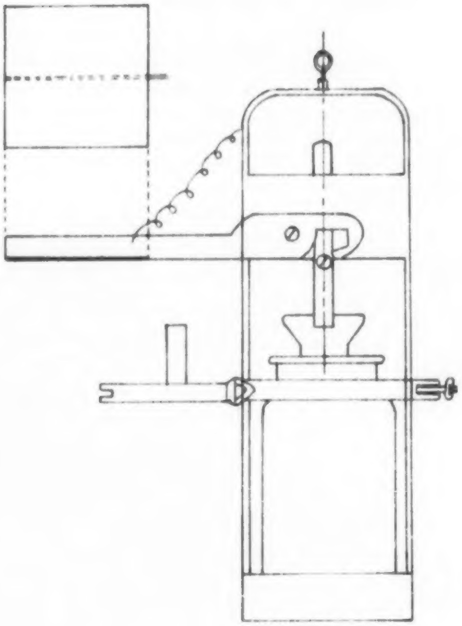

FIGURE 4

3 A commercial sampler is available which is an evacuated sealed tube with a capillary tip. When a lever on the support rack breaks the tip, the tube fills. Other samplers exist with a lever for pulling the stopper, while another uses an electromagnet.

VI DATA RECORDING

A Information generally includes: date, time of collection, temperature of water, location of sampling point, and name of the sample collector. Codes are often used. The location description must be exact enough to guide another person to the site. Reference to bridges, roads, distance to the nearest town may help. Use of the surveyors' description and maps are recommended. Mark identification on the bottles or on securely fastened tags. Gummed tags may soak off and are inadvisable.

B While a sanitary survey is an indispensable part of the evaluation of a water supply, its discussion is not within the scope of this lecture. The sample collector could supply much information if desired.

VII SHIPPING CONDITIONS

A The examination should commence as soon as possible, preferably within one hour. A maximum elapsed time between collection and examination is 30 hours for potable water samples and 8 hours for other water samples (collection 6 hours and laboratory procedures 2 hours). Standard Methods (13th Edition) recommends icing of samples between collection and testing.

VIII PHOTOGRAPHS

A photograph is a sample in that it is evidence representing water quality. Sample collectors and field engineers may carry cameras to record what they see. Pictures help the general public and legal courts to better understand laboratory data.

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Descriptors: Equipment, Microbiology, Sampling, Water Sampling

WATER QUALITY SURVEYS

ROLE OF BACTERIOLOGIST

I INTRODUCTION

The determination of bacteriological quality of water should include active participation of the bacterial analyst in all phases of the survey, from the planning stages through and including the performance of the survey and the final preparation and presentation of the finished report.

The principal bacteriologist may be a professional bacteriologist serving as the principal survey bacteriologist, a bacteriological consultant, or, in practice, these duties sometimes may be assumed by a qualified engineer or scientist nominally representing some other professional discipline.

Chief responsibilities of the principal bacteriologist in a survey are summarized in the following paragraphs.

II IDENTIFICATION OF BACTERIOLOGICAL IMPLICATIONS OF SURVEY OBJECTIVES

A Through consultation with survey management and review of survey objectives, he determines what specific pollution problems are related to bacteria.

B A determination is made of the kind of bacteriological information which must be developed in order to meet survey objectives.

C The related chemical and physical tests are identified in terms of their expected need with respect to interpretation of bacteriological data.

III DETERMINATION OF PROCEDURES

In order to develop the kind of bacteriological information required to meet survey objectives, the following decisions must be made, and pertinent information obtained:

A The specific indicator organisms used to detect and evaluate pollution must be selected.

B The methods used for their quantitative measurement must be determined.

C Consultations must be included with respect to bacteriological sampling.

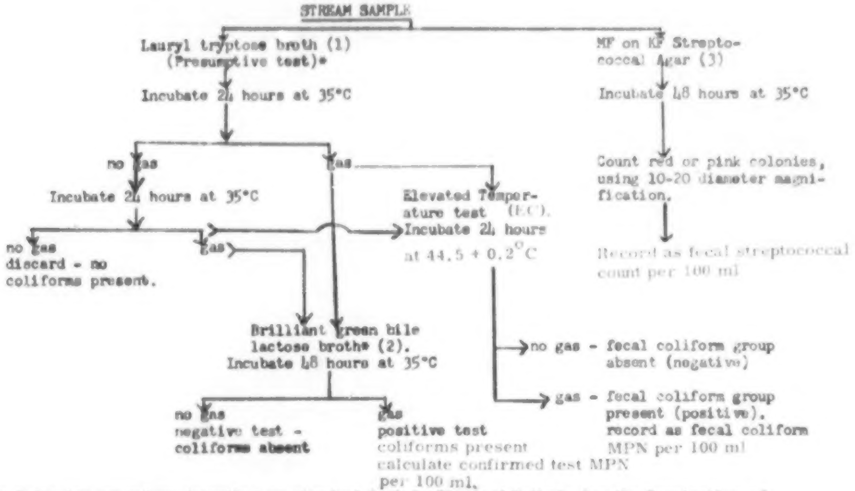
  1 The sample points must be established.

  2 The frequency of bacteriological testing at each point must be determined.

  3 The location of proposed sampling stations with reference to assumed influences on bacterial levels must be reviewed. These influences may be alleged pollution sources, entrance of diluting waters, drainage areas, or, in some cases, industrial drainage or outfalls.

D Any existing data on bacterial densities, as well as water flow velocity and volume should be obtained and studied.

E Based on the sampling plan and determinations to be made, a protocol of bacteriological sample examination is developed. Figure 1 is an example of such a protocol, used in a recent water pollution survey.


STREAM SAMPLE
Lauryl tryptose broth (1) (Presumptive test)*
Incubate 24 hours at 35°C
no gas
gas
Incubate 24 hours at 35°C
no gas discard - no coliforms present.
gas
Elevated Temperature test (EC). Incubate 24 hours at 44.5 + 0.2°C
Brilliant green bile lactose broth* (2). Incubate 48 hours at 35°C
no gas negative test - coliforms absent
gas positive test coliforms present calculate confirmed test MPN per 100 ml.
no gas - fecal coliform group absent (negative)
gas - fecal coliform group present (positive). record as fecal coliform MPN per 100 ml
MF on KF Streptococcal Agar (3)
Incubate 48 hours at 35°C
Count red or pink colonies, using 10-20 diameter magnification.
Record as fecal streptococcal count per 100 ml

* Indicates procedure described as standard test in Standard Methods for the Examination of Water and Wastewater, 13th ed. (1971)

(1). Lactose broth may be substituted for lauryl tryptose broth.
(2). Eosin Methylene blue or Endo agar may be substituted for the brilliant green bile lactose broth.
(3). The KF Agar pour plate count may be used interchangeably with the MF colony counting technique.

Figure 1

IV REVIEW OF BACTERIOLOGICAL PLANS

The bacteriological plan is reviewed for adequacy to accomplish the objectives of the survey, based on a "paper review" and on preliminary samples which should be examined at the selected sampling stations if at all feasible.

A Sufficient qualified personnel for the laboratory load should be provided. Estimates of work loads are shown in Part 2 of this chapter.

B The detailed methods for all procedures are reviewed with all personnel responsible for collection and handling, transmission to the laboratory, and with the laboratory personnel responsible for sample testing procedures.

C The duties assigned to all personnel must be reviewed with all concerned, work scheduled, reviewed and understood by all. Care must be taken to assure that assigned work is consistent with training, ability, and experience of individuals concerned.

D Location and quality of the laboratory facilities must be reviewed to ascertain that sample testing procedures will be in accordance with established plan, standard procedures, and to assure that adequate work space is available.

E Laboratory equipment and supplies must be adequate for the expected work loads. Glassware and other supplies which must be used on a rotational basis should, in general, be $3\frac{1}{2}$ - 4 times the expected daily need.

V REVIEW OF LABORATORY OPERATIONS

After the established daily work of the survey has gotten well underway, the principal bacteriologist should review operations based on the plan established as the final plan for survey operations. Typical check points include:

A Review of sample collection and handling procedures and assurance that there is minimum delay between collection and starting testing procedures on individual samples.

B Evaluation of Laboratory Work

1 Determination of whether the methods actually used are in conformance with plans.

2 Elimination or justification of deviations from planned procedures.

3 Problems which have arisen during the operations are discussed, particularly with reference to matters not anticipated when planning the survey.

4 Data are reviewed to determine validity for use in the survey, in terms of reliability of results.

a When membrane filter or plate count methods are used, check the correlation between occasional duplicate or triplicate plate count for reproducibility.

b When multiple dilution tube methods are used, check the percentage of the usual codes, according to the method described by Woodward and Walton (JAWWA, 1957; 49:1060-1068, "How Probable is the Most Probable Number?")

Table 1. STATISTICAL EXPECTANCY OF POSITIVE TUBE COMBINATIONS FOR A SERIES OF 5 PORTIONS PLANTED IN EACH OF 3 DECIMAL DILUTIONS

Expectancy group	Positive tube combinations constituting group							Frequency of occurrence	
								By group %	Cumulative %
Group I (Frequent)		100	200	300	400	500	550	67.5	67.5
						510	551		
						520	552		
						530	553		
						540	554		
Group II (Common)		110	210	310	410	511		23.6	91.1
					420	521			
						531			
						541			
						542			
Group III (Uncommon)	001		101	201	301	401	501	7.9	99.0
	010		120	211	311	411	502		
	020			220	320	421			
					330	430	522		
						431	532		
						440	533		
							543		
							544		
Group IV (Rare)	All other positive tube combinations							1.0	100.0

Ref.: Richard L. Woodward and Graham Walton. 1957.

Table 2. ACTUAL POSITIVE TUBE COMBINATIONS CLASSIFIED BY EXPECTANCY GROUP PLANT A. RAW WATER COLIFORM TEST

Positive tube combination (5 portions planted in each of 3 decimal dilutions)	Number of times found	I Frequent	II Common	III Uncommon	IV Rare
100	1	1			
110	1		1		
200	2	2			
220	1			1	
300	6	6			
301	1			1	
310	3		3		
400	8	8			
401	1			1	
410	12		12		
411	2			2	
412	1				1
420	4		4		
430	2			2	
440	1			1	
500	13	13			
510	11	11			
511	2		2		
512	1			1	
520	9	9			
521	2		2		
530	9	9			
531	3		3		
540	3	3			
541	5		5		
542	1		1		
550	2	2			
551	1	1			
Total No.	108	65	33	9	1
Percent of total	100. 0	60. 3	30. 5	8. 3	0. 9
Cumulative percent	100	60. 3	90. 8	99. 1	100. 0

Ref.: Richard L. Woodward and Graham Walton. 1957.

c Data sometimes show apparent anomalies which necessitate additional test procedures or special tests on selected samples in order to account for seemingly unusual results or to establish the validity of the results being obtained.

VI INTERIM REVIEW OF DATA

It is essential that periodic review of data be conducted during the survey. It is suggested that such reviews be conducted daily during the initial stages of the survey and thereafter at weekly or biweekly intervals.

A Review can reveal the need for changes in procedures to meet the objectives of the survey.

B Changes needed in sampling plan can be revealed. According to the type of results being obtained, it may be necessary to add further sampling stations, to delete certain stations, or to modify the schedule of sample collection.

C Need for additional test procedures may be demonstrated.

D Interim data may show that certain test procedures are producing data of no value in meeting survey objectives; such procedures can be dropped.

VII FINAL REVIEW OF DATA AND PREPARATION OF REPORT

Development of the survey plan, performance of the planned operations to meet survey objectives, and the final data analyses and preparation of report are interdependent activities.

There are many techniques available for summarizing and presenting data for interpretation of findings. This topic is discussed at greater length in the outline titled "Presentation and Interpretation of Bacteriological Data."

Laboratory Operations

I INTRODUCTION

Part 1 of this outline has been concerned with planning the bacteriological aspects of a survey. With this part of the outline, certain specific recommendations are presented with respect to personnel qualifications and work schedules, desired location and features of laboratory facilities, and the care and handling of samples prior to starting test procedures.

II PERSONNEL

A Skill Levels

1 The principal bacteriologist should have at least 3 - 5 years of professional experience in the sanitary bacteriology of water. The duties of the principal bacteriologist, who may or may not personally perform the laboratory procedures, have been identified in Part 1 of this outline.

2 Subordinate bacteriologists preferably should have at least 1 - 2 years of working experience in water bacteriology.

The laboratory bacteriologist is responsible for the correct testing of samples according to predetermined plans and for preparation of accurate records of results, in an orderly manner. Nonprofessional laboratory assistants, if employed, must be closely supervised in all duties undertaken.

3 Nonprofessional laboratory assistants may be needed. In a short-term, highly intensive survey, the writer is reluctant to employ any but experienced laboratory helpers. Such experience can best be gained in a fixed laboratory where close supervision and direction are available for routine operations than would be possible with the short-term intensive survey operations.

Duties of nonprofessional laboratory assistants include washing and sterilization of glassware, preparation of sample bottles and related supplies, preparation and maintenance of culture media supplies, and related duties.

B Work Loads

1 Based on the assumption of utilization of highly skilled, fast, laboratory workers, the bacteriologist-consultants of many water quality surveys have recommended a maximum of 20 coliform determinations per man-day of bacteriology laboratory service. This includes media preparation and other laboratory support service.

2 Additional tests may require adjustment of work loads.

a Fecal coliform tests require a slight addition of time. For planning purposes however, coliform tests plus fecal coliform tests have been considered to permit the 20-samples per man-day. Probably 16 samples per man-day is more realistic.

b The addition of fecal streptococcus tests to the coliform and fecal coliform tests requires major readjustment in the number of daily samples. With this series of tests, a maximum of 10-14 samples can be examined per man-day of laboratory service.

3 When considering above work loads, the customary 8-hour day may not be possible.

For a short-term, highly intensive, survey there usually is little difficulty in operating on this basis, provided that personnel are motivated to the task at hand. With long-term investigations, personnel levels should be held at such levels as to permit normal work-days and work-weeks with off-duty days provided at periodic intervals.

III LABORATORY FACILITIES

A Location

The requirement for prompt examination of samples after collection demands attention to the location of laboratory facilities.

1 With suitable transportation arrangements it may be possible to perform examinations in established water bacteriology laboratories. This is to be preferred if at all possible. Recently, increasing attention has been given to air transport of samples as a means of resolving this problem.

2 With limitations on available transportation it more commonly is necessary to establish a temporary laboratory. This may be temporary space in established laboratories, as in local or hospital laboratories, or it may be necessary to use a mobile trailer laboratory.

3 It is not practiced to conduct the entire bacteriological examination of water in stream surveys under field conditions, even with membrane filter methods. In some cases, however, it may be necessary to inoculate samples into primary tube media or on membrane filters in the field. In such samples, temporary incubation must be provided (no icing of inoculated samples) for transport of the sample to the laboratory where the remainder of the incubation and subsequent laboratory procedures are performed.

B Space

The bacteriology laboratory requires provision for several functions, each of which requires a significant amount of space. To give assurance that adequate space is provided, the following functions must be considered:

1 Bench space

Bench space of the type used for chemical determinations is suitable for bacteriological work. Unless the number of bacteriological samples is quite small, the bench space should be reserved for this function. About six lineal feet of bench space per worker is a minimum allowance.

2 Preparation area

If the number of daily samples is low, it may be feasible to make and dispense culture media, prepare sample bottles, etc., at the laboratory bench. If the work load is large, one general area should be reserved for media preparation, washing and sterilization of glassware, and other supporting functions.

3 Incubation and sterilization

Space must be provided for certain fixed laboratory equipment, such as washing equipment, dry sterilizer and autoclave, water bath, and incubator, according to the work load anticipated for the survey.

IV SUPPLIES

A Source

In survey planning, consideration should be given to preparing all culture media and reusable supplies in a central laboratory, and transporting such supplies to the field laboratory site. The practice frequently is more economical than efforts to provide such support at a field site.

B Sample Bottles

1 All sample bottles must be clean, sterile, and free of substances unfavorable to bacterial survival. Although a wide variety of containers is acceptable, the preferred form is a glass-stoppered, wide-mouth borosilicate glass bottle, having about 250 ml capacity.

2 Some samples, such as effluent from some waste treatment plants, may contain residual chlorine. In such cases, enough sodium thiosulfate is introduced into the clean sample before sterilization to give 100 mg/l thiosulfate in the sample. For example, add 0.2 ml of a 10% solution of sodium thiosulfate to a 250 ml bottle such as described above. This will dechlorinate samples containing up to about 23 mg chlorine per liter. The bottle is dry or moist heat sterilized following introduction of the sodium thiosulfate.

Laboratory tests have shown that this amount of sodium thiosulfate has no adverse effect on the survival and growth of coliform bacteria or fecal streptococci.

V SAMPLE COLLECTION AND HANDLING

A Collection

1 In many cases, sampling is limited to surface grab samples. The opened bottle is plunged into the water to a depth of about one foot. The sample bottle never should be filled more than one-half to three-fourths full. This is to facilitate mixing by shaking when laboratory tests are started.

2 If but one sample is taken from each station at the selected intervals, it usually is collected from near the center of the main channel of flow. Preliminary tests may demonstrate need for multiple samples from each station. In this case, it may be necessary to collect samples from predetermined points across the body of water and/or at designated depths. Such samples are not composited and retain their identity as single samples throughout the laboratory testing procedure.

3 The sample collection is made in such a manner as to insure obtaining a sample representative of the source. Thus, if sampling is from a boat, the bottle should be dipped into the upstream side of the boat, with the mouth of the bottle also directed upstream. If there is no discernible current, then the bottle is moved through the water in a direction away from the hand holding the bottle.

4 If depth sampling is indicated, it is necessary to use special depth samplers, and open them at the desired depth.

B Identification

1 All samples must be immediately and fully identified at the time of collection, including at least the sample location, and the date and time of sampling. Many workers use a supplemental sheet to record temperature, pH and other data.

2 The sample tag should be affixed to the sample bottle. If the bottle has permanent identifying marks, a supplemental sheet may be used to identify the sample.

3 Wax pencils are not satisfactory. Indelible pencils are preferred. Any marking material that will run if wetted will be unsatisfactory.

C Care in Transit

1 Time between sample collection and starting tests

a The test procedures should ideally be run immediately, or if this is not feasible, should preferably be started within 1 hour after sample collection; it never should exceed 8 hours (six hours transit time and two hours laboratory).

b With some samples, such as those from heavily polluted streams, even greater limitation on this interval is necessary.

2 Temporary storage

With temporary storage of samples, "Standard Methods" now recommends the practice of icing samples prior to examination.

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Descriptors: Personnel, Planning, Laboratory Equipment, Laboratory Tests, Microbiology, Sampling, Surveys, Water Quality

INTRODUCTION TO STATISTICS

Part I

Summarization of Data

I INTRODUCTION

The body of statistical methods comprises two groups generally called descriptive statistics and statistical inference. Descriptive statistics encompasses primarily graphical techniques for the presentation of data while statistical inference is basically a mathematical approach to the problem of inferring from a part to the whole.

II BASIC GRAPHICAL TECHNIQUES

In this section we consider the non-mathematical techniques of summarizing a set of data in order that the meaningful information can be extracted from it.

Consider the data in Table 1 which represent aqueous fluoride ion concentrations determined by a colorimetric procedure.

TABLE 1

F^-, mg/liter		
0.68	0.77	0.85
0.71	0.79	0.85
0.72	0.80	0.87
0.74	0.81	0.90
0.75	0.82	0.91
0.77	0.83	0.95

A Frequency Table

As the first step in summarizing a set of data we form a frequency table (See Table 2). To construct the table the data are divided into a number of intervals of equal length called class intervals and the number of results falling into each interval is recorded in the "frequency" column.

The number of class intervals chosen is arbitrary. However, it is a rule of thumb to choose the length of the class interval so that 7 to 15 intervals will include all the data under consideration.

TABLE 2

Class Interval	Frequency
.65 au* .70	1
.70 au .75	3
.75 au .80	4
.80 au .85	4
.85 au .90	3
.90 au .95	2
.95 au 1.00	1

* and under

B Frequency Polygon

As a further step we can graph the frequencies recorded in the frequency table. One way of doing this is to plot the frequency along the ordinate (vertical axis) and the midpoint of the class interval along the abscissa (horizontal axis). The line connecting the plotted points in Figure 1 form a frequency polygon.

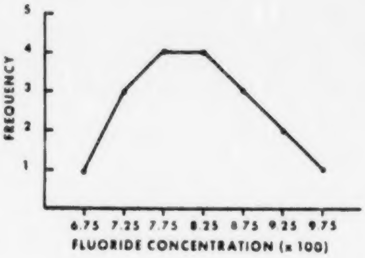

FIGURE 1

C Histogram

Another method of graphing the frequency information is to construct a histogram as shown in Figure 2. The histogram is a two dimensional graph in which the length of the class interval is taken into consideration. Each class interval becomes the base of a rectangular bar whose height is equal to the corresponding frequency.

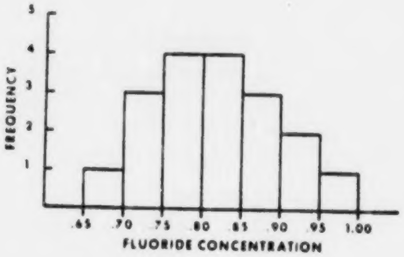

FIGURE 2

D Histogram of Relative Frequency

The histogram can be a very useful tool in statistics especially if we convert the given frequency scale to a relative scale such that the sum of all the ordinates equals one. Thus, each ordinate value is derived by dividing the original frequency by the number of observations in the sample. For the data in Table 1 we would divide each of the frequency values in Table 2 by 18.

The advantage of constructing a relative frequency histogram like the one in Figure 3 is that we can interpret areas under the histogram as probabilities provided we assume a scale on the abscissa such that each class interval is of unit length. Then the probability that a given value will fall in any one interval is the area under the histogram in that interval.

For example, the probability that a value will fall between .70 and .75 is equal to the area under the histogram in that interval which is 3/18 × 1 = 1/6.

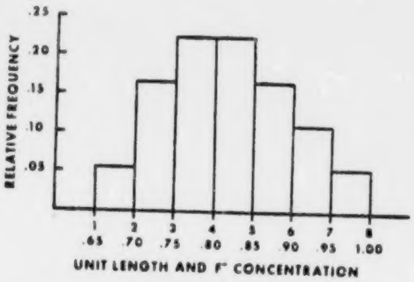

FIGURE 3

E Cumulative Frequency Distribution

From a frequency table (See Table 2) we can construct a cumulative frequency table as shown in Table 3. The cumulative frequency table gives the number of observations less than a given value. The cumulative frequency values in Table 3 are found by summing the frequencies in Table 2.

TABLE 3

F^- Conc.	Cum. Frequency
under .70	1
" .75	4
" .80	8
" .85	12
" .90	15
" .95	17
" 1.00	18

If we convert the cumulative frequency values to relative frequencies as we did to construct the relative frequency histogram, we can plot the relative values to form a cumulative frequency curve as shown in Figure 4. The probability that a result will fall below a given value can be read from the cumulative frequency curve. For example, to find the probability that a result will be less than

0.85 mg/liter we read up to the curve at the point x = .85 and across to the value .67 on the probability axis.

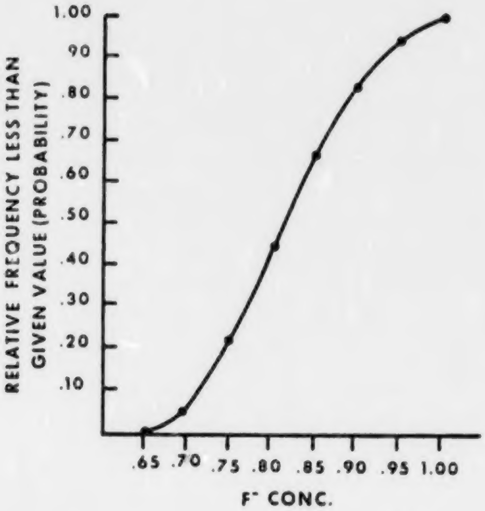

FIGURE 4

INTRODUCTION TO STATISTICS

Part II

Measures of Central Tendency and Dispersion

I INTRODUCTION

From a practical point of view, statistical inference is the application of mathematical tools to analyze and interpret the results of investigations in the physical and social sciences with respect to the hypotheses under investigation. The need for a statistical approach rests on the well-founded assumption that any such result contains an inherent component of error or random variation which introduces uncertainty into any conclusions which may be drawn from the observed results.

A Mathematical Distributions

It is further assumed that the error components can be described by a mathematical function called a probability distribution. Knowledge of the relevant type of theoretical distribution provides us with the means of assessing the uncertainty (risk of being incorrect) in conclusions drawn from observed results.

B Population Parameters

The concept of distribution pertains to the totality of possible results which would be observed in an infinite number of repetitions of an experiment or investigation. This theoretically infinite number of results is called a population. Any measurable characteristic of a population distribution is called a parameter. For each type of distribution there exists a characteristic set of parameters. Specific values for the parameters of a given distributional form define a particular distribution of that type. In almost every case the entire population can never be observed and therefore the values for the parameters are unknown. Population parameters are denoted by lower case Greek letters.

C Sample Statistics

The observed results from a particular investigation are called a sample of the relevant population. In almost every case the sample is a subset of the population. A statistic is a function of the sample points (i.e., observed results) which usually estimates a population parameter. Sample statistics are denoted by English letters.

D Classification of Statistical Measures

It is implied above that the type of population distribution determines the particular function of the observed results (i.e., sample statistic) which will provide the "best" estimate of the corresponding population parameter. For this reason it is advantageous to assume that any given sample came from a population with a specified form of distribution. The use of graphical techniques to indicate the form of distribution should now be apparent.

In most situations the distributions or frequency functions of interest have a single peak and characteristic dispersion of area about this peak. Parameters which locate the distribution (by the peak, center of gravity, midpoint, etc.) are called location parameters and the corresponding sample statistics are classified as measures of central tendency. Parameters which describe the dispersion of area are called scale parameters and the corresponding sample statistics are classified as measures of dispersion. Ideally, one would like to encounter only distributions for which one measure of central tendency and one measure of dispersion summarize all of the relevant information in the sample about the population.

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II MEASURES OF CENTRAL TENDENCY

In this section and the next we define and lay out the computational format for the more commonly used measures in statistics. We let x_i denote a typical observed result so that $[x_1, x_2, \ldots, x_n]$ represents a sample of n observations.

A The Mean

The most commonly used measure of central tendency is the mean or arithmetic average. We denote the sample mean by $\bar{x}$ and the population mean, of which $\bar{x}$ is an estimate, by μ. The computational formula is:

$$\bar{x} = \frac{\Sigma x_i}{n}$$

For the fluoride data in Table 1, we calculate the sample mean as follows:

$$\bar{x} = \frac{\Sigma x_i}{n} = \frac{14.52}{18} = 0.807$$

TABLE 1

F^-, mg/liter		
0.68	0.77	0.85
0.71	0.79	0.85
0.72	0.80	0.87
0.74	0.81	0.90
0.75	0.82	0.91
0.77	0.83	0.95

B The Median

Another common measure of central tendency is the median. The median is the midpoint of an array of numbers (ordered according to value). To find the sample median we need only arrange the data in ascending or descending order and pick the middle value. When there is an even number of observations take the average of the two middle values. For the data in Table 1 the median is 0.805.

C The Mode

The mode is another measure of central tendency, although it is of little practical importance. The mode is the most frequently occurring value. Therefore, the population mode is the value corresponding to the peak of the frequency distribution curve. Frequency distributions with more than one peak are called multimodal. In a symmetrical frequency curve the mean, median, and mode are all equal.

III MEASURES OF DISPERSION

A The Standard Deviation

As with measures of central tendency, there are several measures of dispersion, the most common of which is the standard deviation. We denote the sample by s and the population value of which s is an estimate by σ. The computational formula is:

$$s = \sqrt{\frac{\Sigma (x_i - \bar{x})^2}{n - 1}}$$

However, computation using this formula is tedious and it is relatively simple to show the following relationship:

$$s = \sqrt{\frac{\Sigma (x_i - \bar{x})^2}{n - 1}} = \sqrt{\frac{n \Sigma x_i^2 - (\Sigma \bar{x}_i)^2}{n (n - 1)}}$$

The derived formula is preferable because of its adaptability to the desk calculator. We calculate the sample standard deviation of the fluoride data in Table 1 as follows:

$$s = \sqrt{\frac{(18)(11.8048) - (14.52)^2}{18 (17)}}$$

$$= \sqrt{\frac{212.4864 - 210.8304}{306}}$$

$$= \sqrt{\frac{1.656}{306}} = \sqrt{0.0054} = 0.073$$

B The Variance

The sample value s^2 is referred to as the sample variance and is merely the square of the standard deviation. Its formula is:

$$s^2 = \frac{\Sigma(x_i - \bar{x})^2}{n - 1}$$

The population variance is represented by σ^2. Its formula is:

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{n}$$

This is the same as the formula for s^2 except we use the true population mean μ rather than its estimate $\bar{x}$ and divide by n instead n - 1.

Obviously in calculating the sample variance the true mean is not known and we, therefore, use the estimate of the mean from the data. In calculating the sample mean and then using it to calculate the variance of the same data we lose what is called a degree of freedom. It can be shown that the estimate of the variance must be based upon the sum of independent squared terms. We average our values over n - 1 because this is the number of independent squared terms that we will have when using a mean that has been estimated from the sample. In line with above, we should draw the distinction between the variance of the sample and the sample variance. The variance of the sample, we would calculate for its own sake and divide by n. However, we calculate the sample variance as an estimate of the population variance and divide by n - 1. There is no practical use of the variance of the sample, therefore, we always calculate the sample variance dividing by n - 1 which we shall call the number of degrees of freedom in our sample. As a rule, in any calculation, for every parameter that must be estimated, one degree of freedom (d. f) is lost.

C The Range

The range is also used as a measure of dispersion. It is the difference between the highest and lowest values in a set of data.

$$R = \max(x_i) - \min(x_i)$$

For the data in Table 1 the range is then:

$$R = 0.95 - 0.68 = 0.27$$

A rough estimate of s can be made by dividing the range of the sample by the square root of n, the number of observations, when n is small.

$$s \simeq \frac{0.27}{\sqrt{18}} \simeq \frac{0.27}{4} \simeq 0.067$$

The use of the range is limited to instances where the labor of computing the standard deviation is impractical.

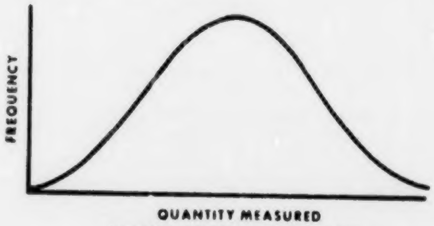
IV THE NORMAL DISTRIBUTION

A The most important theoretical distribution in statistics is the familiar bell-shaped normal distribution which is symmetric about its peak (See Figure 1). The following assumptions give rise to this distributional form:

1 Values above or below the mean are equally likely to occur.

2 Small deviations from the mean are extremely likely.

3 Large deviations from the mean are extremely unlikely



NORMAL DISTRIBUTION CURVE

FIGURE 1

The normal distribution is completely defined by its mean, μ, and its standard deviation σ in the following manner:

1 The area under the normal curve between μ minus σ and μ plus σ is 68 percent of the total area, to the nearest 1 percent.

2 The area under the normal curve between μ minus 2σ and μ plus 2σ is 95 percent.

3 The area under the normal curve between μ minus 3σ and μ plus 3σ is 99.7 percent of the total area, to the nearest 0.1 percent.

If a frequency curve is a good approximation to the normal curve, these characteristics of the normal curve can be used to find information about the frequency distribution.

V TRANSFORMATION OF DATA

A Skewed Distribution

In some areas of investigation one often encounters distributions which are not symmetric. For example, distributions of bacterial counts are often characterized by many more extremely high counts relative to the median than extremely low counts. The frequency curves of these distributions have a long right tail as shown in Figure 2. Distributions of this type display positive skewness.

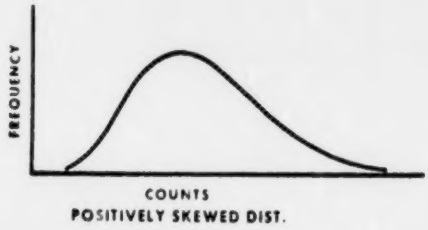


FIGURE 2

B Logarithmic Transformation

For many reasons, both practical and theoretical, we prefer to work with symmetric distributions like the normal curve. Therefore, it is desirable to transform skewed data in such a way that a symmetric distribution results, resembling the normal. One way of deriving an approximately normal distribution from a positively skewed distribution is by expressing the original data in terms of logarithms. An artificial sample of coliform counts and their logarithms are shown in Table 2. Comparison of the frequency tables for the original data and the logs (Table 3 and Table 4, respectively) clearly shows that the logarithms more closely approximate a symmetric distribution.

TABLE 2

Coliform MPN	MPN/100 ml log MPN
11	1.041
27	1.431
36	1.556
48	1.681
80	1.903
85	1.929
120	2.080
130	2.114
136	2.134
161	2.207
317	2.501
601	2.779
760	2.881
1020	3.009
3100	3.491

TABLE 3

Class Interval	Frequency (MPN)
0 au 400	11
400 au 800	2
800 au 1200	1
1200 au 1600	0
1600 au 2000	0
2000 au 2400	0
2400 au 2800	0
2800 au 3200	1

TABLE 4

Class Interval	Frequency (log MPN)
1.000 au 1.300	1
1.300 au 1.600	2
1.600 au 1.900	1
1.900 au 2.200	5
2.200 au 2.500	1
2.500 au 2.800	2
2.800 au 3.100	2
3.100 au 3.400	0
3.400 au 3.700	1

C Measures of Central Tendency of Skewed Distributions

If the logarithms of data from a positively skewed distribution are approximately normally distributed, we say that the original data have a log-normal distribution. The best estimate of central tendency of log-normal data is the geometric mean defined as:

$$\bar{x}_g = \sqrt[n]{(x_1)(x_2)\ldots(x_n)}$$

Notice that

$$\log \bar{x}_g = \frac{\Sigma(\log x_i)}{n}$$

so that the geometric mean of the original data is equal to the antilog of the arithmetic mean of the logarithms. It is of interest that the population geometric mean is equal to the population median. Thus, in the case of data from a skewed distribution, the sample median is a better estimate of central tendency than the arithmetic mean of the original data. For the coliform data in Table 2 we calculate:

$$\log \bar{x}_g = \frac{\Sigma(\log x_i)}{n} = \frac{32.737}{15} = 2.1825$$

$$\bar{x}_g = \text{antilog}\,(2.1825) \simeq 152,$$

$$\text{median} = 130,$$

$$\bar{x} = \frac{\Sigma x_i}{n} = \frac{6632}{15} = 442.$$

This outline was prepared by John H. Parker, Former Statistician, Analytical Reference Service, Training Program.

Descriptors: Frequency Analysis, Histograms, Statistical Methods, Statistical Models, Statistics, Variability

PRESENTATION OF DATA

I INTRODUCTION

The data collected during any water quality survey must ultimately be reduced to some simple, expressive form for presentation Ideally, the data presentation should be easily understood by the least trained personnel as well as by the trained professional. The data presentation alone may determine the response to the survey.

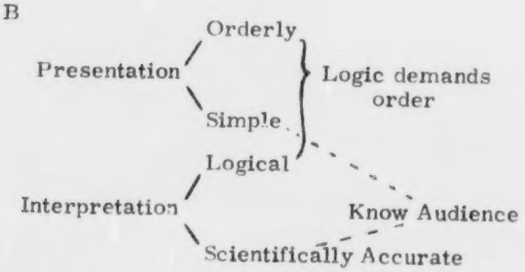
II ANALYSIS OF DATA

A Before one analyzes any information, the data must be set into logical order and the analyst must present the information to himself.

In developing this order, the analyst should keep in mind the objectives of the survey, and the variables encountered during the study.

The analyst's "private" presentation to himself enables him to evaluate or interpret the data in a logical and scientific manner.

B



III PRESENTATION OF DATA

This is one of the most important areas of report writing and unfortunately is often neglected or overlooked.

A Common Methods of Presenting Data

1 Single value

2 Single value plus related information

3 Graphic methods

B Single Value Approach

1 Methods commonly used

a Arithmetic mean

b Geometric mean

c Median value

d Mode value

2 Advantages of using single value approach

a Economical - engineering design criteria is often based on average values. In some instances minimum and maximum values are more critical.

b Instrumentation - advanced analytical techniques and instrumentation are accounting for the collection of tremendous amounts of information. The tendency is to compile this information on computers and use sophisticated statistical methods to arrive at representative mean values.

c Audience - since some readers (local, state or federal legislators) do not have the technical background or (especially), interest to read through many pages of numbers, it becomes convenient to summarize the numerous observations by using single representative values. In effect, the analyst is a salesman, his report being his product.

C Single Value Plus Related Information

1 Methods commonly used

a Single value plus maximum or minimum value

b Single value plus percentage range (usually 80%) of survey or laboratory results

c Single value plus standard deviations from the mean (depending upon degree of confidence required)

d Distribution and frequency distribution tables

2 Advantages of using single value plus related information.

  a Eliminates some misinterpretation caused by extremely high or low results that occur rarely.

  b Gives an idea of what type of distribution is occuring (normal, skewed, etc.)

  c Average values are extremely useful but it is usually the maximum or minimum values that pose serious problems.

D Graphic Methods

One of the easiest, most attractive, and most efficient ways to present numerical information is by use of graphs.

1 Graphs commonly used

  a Normal graph paper (Cartesian Coordinate paper) - This is the most generally used graph paper and is most useful in obtaining an idea of the trend the numerical observations are taking. This type of paper is most helpful in preliminary plotting of information to decide if semi-log, log-log or other types of functional graph paper would be more desirable to use.

  b Functional graph paper (semi-log, log-log) - Use of this paper allows for the plotting of results in straight line fashion that would appear on normal graph paper (Cartesian Coordinate paper) as a curved line.

  c Arithmetic or geometric (log) probability paper - this type of graph plots the normal frequency curve (Bell shaped curve) as a straight line.

  d Nomograph - Used where complex equations or complicated mathematical manipulations and calculations are involved. Nomographs have been developed for numerous applications.

  e Special graphs

    1 3-dimensional diagrams

    2 pie-diagrams

    3 histograms, etc.

2 Desirable characteristics of graphs

  a Fairly simple

  b Be able to stand alone

  c Graphs should be designed so they may be easily reproduced.

IV SUMMARY

The presentation of information is one of the first steps in report writing. It is one of the most important parts of any report and if properly handled can produce a report that is brief, understandable and useful to the reader. The manner in which results are presented (written or oral) may serve as a basis for judgment of the whole survey.

This outline was prepared by P. F. Atkins, Jr., Sanitary Engineer, formerly with Training Activities, EPA.

Descriptors: Data Collections, Data Handling, Statistics

WATER QUALITY SURVEYS

PRESENTATION AND INTERPRETATION OF BACTERIOLOGICAL DATA

I OBJECTIVES

Bacteriological data analysis consists of an orderly assembly and summary of the data obtained in the investigation, in the simplest available manner, leading to demonstration and explanation of the observed results in terms of the initial survey objectives. The topics of summary of data and interpretation of data are discussed separately in this presentation; however these are mutually interdependent activities.

A There are many ways in which data can be summarized and analyzed. The most commonly used procedures are described in this outline.

B A number of criteria apply to the selection of procedures for data analysis:

The analysis must be -

1 Understandable by readers of the report;

2 Consistent with survey objectives;

3 Accurate summary of data; and

4 Representative of technically sound reasoning.

II PRESENTATION OF QUANTITATIVE DATA

A Data on coliform bacteria, fecal coliforms, and fecal streptococci must be presented on a quantitative basis if useful interpretations are to be drawn. Partition counting of coliforms (IMViC typing) or streptococci (biochemical characterization or species identification) should be done on a quantitative basis, through study of a representative number of (100 for example) pure cultures, with determination of percentage of occurrence of each identified variant.

B Development of Expression of Central Tendency

1 Single central value

a Median

The median is determined by assembling all data in an array of ascending or descending order. The median value is the central value, a positional value in the array.

b Arithmetic mean, or average. This is much influenced by individual extremes.

c Geometric mean. (Logarithmic mean)

Many workers prefer use of this value, as it includes all determinate values but minimizes the effects of extremely high or extremely low values.

d In selecting the form of expression of central value, it is necessary to know the methods of calculation required in meeting established standards. For example, geometric mean values often are lower than arithmetic mean values on identical data. If the standards of quality are based on use of arithmetic mean values, it would be necessary to use the arithmetic mean value in the data analysis.

2 Distributions of values

a Data may be calculated and arranged on a distribution table. The method has particular usefulness when the investigation is related to designated standards of water quality.

b The central value may be obtained by one of the above methods, plus showing the maximum value at the designated sampling point.

c The central value may be obtained, plus a percentage range for the data from a single location. Some investigators recommend use of the geometric mean value, plus the range of the middle 80 percent of all values from the data being consolidated. The method permits expression of average water quality (geometric mean) and the maximum and minimum quality demonstrated. Exclusion of the extreme 10 percent of high and low values reduces the danger of misinterpretation by ascribing particular significance to the sporadic extremes of values which may not be truly representative of quality of the water.

C Values Used to Describe Numbers of Organisms

1 Number per 100 ml of water. This is direct use of data obtained in laboratory work, and may be a Most Probable Number value; or it may be a value obtained by membrane filter methods or plate count methods in which a simple calculation is made to relate the number of colonies to the volume of sample tested, computing the number of test organisms per 100 ml.

This form of data expression appears to be most applicable to investigations for compliance with established water quality standards, or to trace pollution indicators when there is a relatively fixed volume of water.

2 In stream surveys where there may be multiple sources of pollution with changes in volume of the receiving water through waste outfalls, or juncture with other streams, it may be necessary to determine the total number of bacteria and trace their fate in the investigation.

Because the numbers of organisms may reach nearly astronomical proportions, and to simplify presentation, consolidated values often are used.

a Bacterial Qualtity Unit (QU) is the number of bacteria passing a given point in one day if the concentration is 1000 organisms per milliliter, and the stream flow is 1 cubic foot per second. One QU is equivalent to 2.45×10^{12} bacteria.

b The Bacterial Population Equivalent (BPE) is another useful tool for consolidating data and representing the total number of organisms in the water being studied. This value does not represent the number of coliform bacteria discharged by an individual but, instead, represents a value obtained by determining the relationship between the number of coliform bacteria passing some downstream point and the number of individuals in the community discharging wastes into the flowing waterbody. The BPE (calculated sewered populations) are derived by converting flows of cfs to flows of 100 ml/day, multiplying by density of coliforms (MPN/100 ml) and dividing the total numbers by 400 billion/day (summer data) or 125 billion/day (winter data).

Summer (15 C. or more):
$\text{BPE} = Q\ (\text{cfs}) \times \text{MPN}/100\ \text{ml} \times (6.1 \times 10^{-5})$

Winter (less than 15 C.)
$\text{BPE} = Q\ (\text{cfs}) \times \text{MPN}/100\ \text{ml} \times (1.95 \times 10^{-4})$

D Methods of Presentation of Data

1 Distribution tables (Table 1)

Table 1. Data Presentation by Showing Distribution of Values

MPN over	2	10	50	100	250	500	750	1000	2500
no. tests	50	49	49	36	31	6	1	1	0
percentage	100	98	98	72	62	12	2	2	0

2 Circles, histograms, or other geometric figures superimposed on maps to show, by relative dimensions, relative values obtained.

3 Graphs

4 Development of mathematical expressions to describe death rate of the organisms.

a The method involved determination of a peak value for coliforms at some point immediately downstream (10 - 15 hours flow time) of the point of discharge.

b The decreasing numbers of bacteria often can be described by the equation

$$Y = A \times 10^{-bt} + C \times 10^{-dt}$$

Where:

Y = the fraction of the peak number of bacteria remaining after the time, t;

A = the fraction of the bacteria out of the peak number which decrease at a rapid rate;

b = the rate at which the "A" fraction of bacteria decrease;

C = the fraction of the bacteria out of the peak number which decrease at a different (lesser) rate; and

d = the rate at which the "C" fraction of bacteria decrease.

The values of the rate coefficient b and d are determined on the basis of the coliform data, using techniques similar to those used for development of the rate coefficient k in the BOD equation. (See Figure 1)

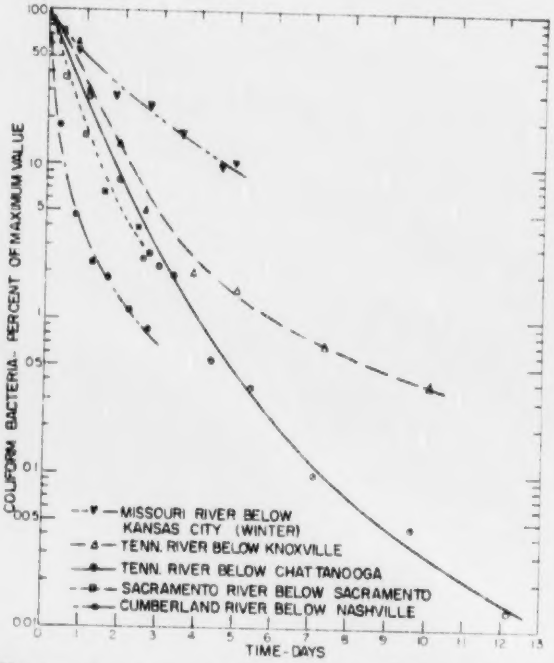


Figure 1. —Rates of coliform decrease below five selected cities.

III INTERPRETATION OF FECAL COLIFORM - FECAL STREPTOCOCCUS RELATIONSHIPS

A Development of Ratios

Using the single central-tendency values from each sampling station in a survey, it often is useful to determine the ratio of fecal coliforms to fecal streptococci. If, for example, at a given station the fecal coliform value is given as 72,000 per 100 ml and the fecal streptococcus value is 16,000, then the fecal coliform/fecal streptococcus ratio is 4.5.

B Interpretation of Ratios

1 When the fecal coliform/fecal streptococcus ratio is greater than 4.0, this is regarded as overwhelming evidence of pollution derived from human origin; or that if the pollution is of mixed origin, the majority of such pollution is of human origin.

2 When the fecal coliform/fecal streptococcus ratio is less than 0.7, this suggests pollution derived predominantly or entirely from livestock or poultry wastes. Feedlots, stockyards, and even stormwater runoff usually produce such ratios.

3 Ratios falling between 4.0 - 0.7 are not quite so certain. To be sure, a ratio of 3.5, for example, would be more suggestive of pollution representing predominantly human origin; and a ratio of 0.9 would be more suggestive of animal origin. A truly "gray-area" of interpretation of these ratios is in the range 2.0 to 1.0.

a When the ratio is in this range, it frequently represents significant mixtures of both human and animal contribution, or

b The source of pollution may be somewhat remote, and due to differences in the rates of disappearance of the two bacterial groups, the original numerical relationships have been obscured.

4 Limitations on interpretation of fecal coliform-fecal streptococcus ratios.

a The ratios have greatest reliability for samples taken not more than 24 hours flow time (or distance) from the origin of the pollution, and

b The ratios must be based on waters in pH range between 4.0 - 9.0.

c Total coliform counts cannot be used in determination or interpretation of ratios with fecal streptococci.

IV PRESENTATION OF QUALITATIVE DATA

A For some determinations, notably demonstrations of pathogenic microorganisms, quantitative methodology either is lacking or is so expensive and time-consuming as to make sure tests completely impractical.

B In surveys involving pollution of intestinal origin, much attention is being given currently to the demonstration of pathogenic bacteria, notably bacteria of the genus Salmonella. Such organisms, when found, are interpreted to represent positive proof of deleterious bacteriological quality of the water, since all members of the genus are disease-causing bacteria.

C The FWQA survey report of the Red River of the North presented data on occurrence of Salmonella as shown in Table 2 and Figure 2.

Table 2. SALMONELLA ISOLATIONS - RED RIVER OF THE NORTH

Date	Station	River mile	Distance from waste source (miles)	Flow time (days)	Total coli/100ml	Fecal coli/100ml	Salmonella isolated
Sept. 1964	Moorhead sewage treatment plant	448	0		Not done	Not done	S. kentucky
	RR 11	436	12	0.5	250,000	64,500	S. kentucky
	RR 12	426	22	1.0	47,600	2,850	S. saint paul
Nov. 1964	RR 9	462	-14		314	49	Absent
	RR 10	441	7	.3	432,000	155,000	S. typhimurium
							S. braenderup
							S. reading
	RR 11	436	12	.4	249,000	85,600	S. braenderup
							S. heidelburg
							S. reading
	RR 12	426	22	.8	68,000	16,800	S. blockley
							S. braenderup
	RR 16	386	62	3.0	6,630	1,610	S. heidelberg
	RR 28	292	7	.3	39,800	2,970	S. reading
	RR 29	286	13	.7	27,800	1,030	S. reading
							S. infantis
Jan. 1965	RR 10	441	7	.2	162,000	61,000	S. chester
							S. thompson
							S. oranienburg
							S. st. paul
	RR 11	436	12	.4	83,500	34,000	S. chester
							S. heidelburg
							S. st. paul
							S. enteritidis
							S. typhimurium
	SH 13	428-1	-	-	650	218	Absent
	RR 14	416	32	1.2	18,600	9,170	S. enteritidis
							S. chester
							S. st. paul
							S. thompson
	RR 16	386	62	3.3	7,800	5,160	S. st. paul
							S. enteritidis
							S. thompson
	RR 18	375	73	4.0	6,140	2,950	S. st. paul
							S. thompson

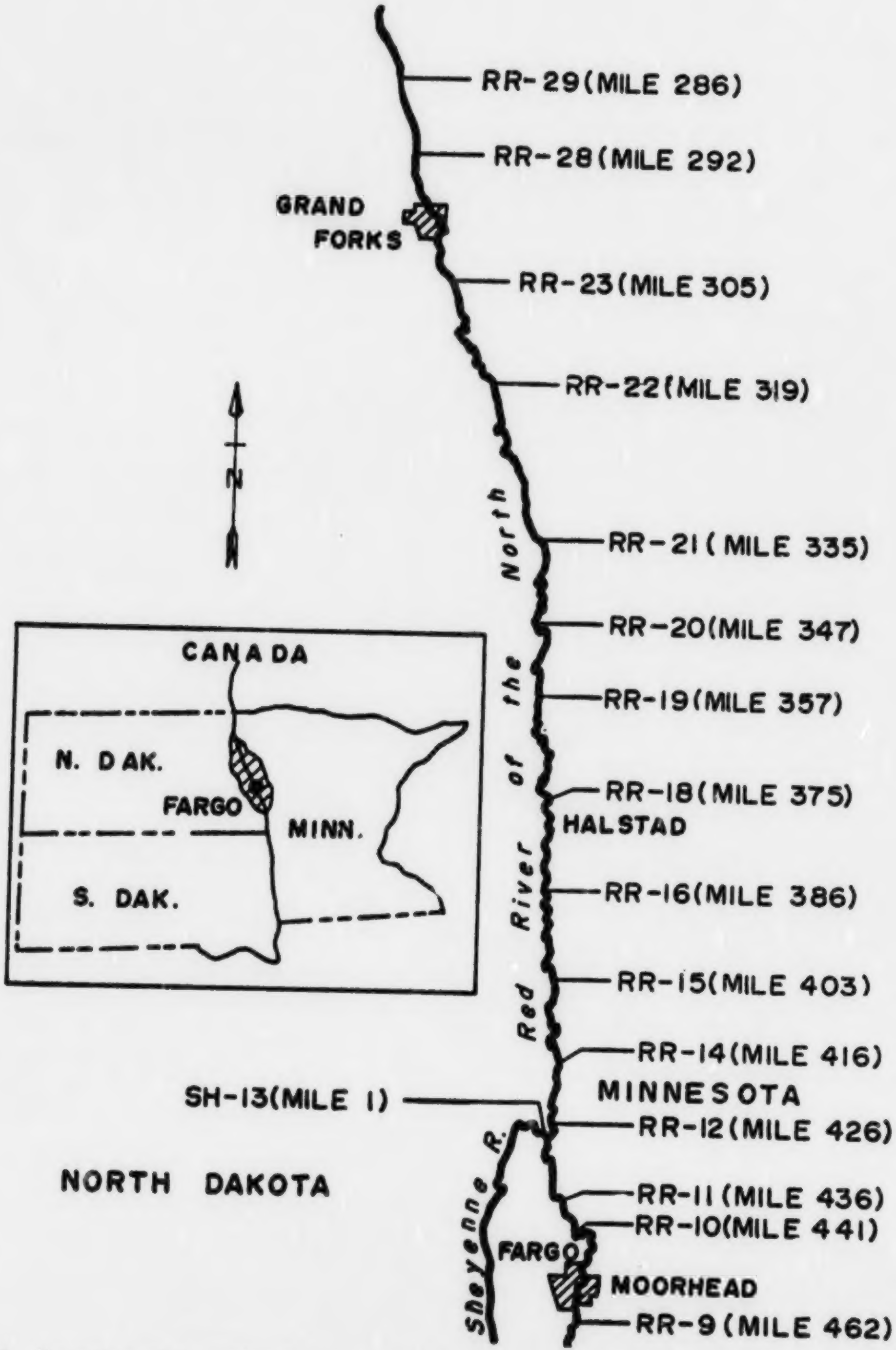

RR-29(MILE 286)
RR-28(MILE 292)
GRAND FORKS
RR-23(MILE 305)
RR-22(MILE 319)
N
RR-21 (MILE 335)
RR-20(MILE 347)
RR-19(MILE 357)
RR-18(MILE 375)
HALSTAD
RR-16(MILE 386)
RR-15(MILE 403)
RR-14(MILE 416)
Red River of the North
SH-13(MILE 1)
MINNESOTA
RR-12(MILE 426)
NORTH DAKOTA
RR-11 (MILE 436)
RR-10(MILE 441)
Sheyenne R.
FARGO
MOORHEAD
RR-9 (MILE 462)
CANADA
N. DAK.
FARGO
MINN.
S. DAK.

Figure 2. Location of Sampling Stations and Station Numbers (River Miles) of Samples from the Red River of the North, North Dakota and Minnesota

V CASE EXAMPLES

The following studies illustrate the ways in which certain pollution surveys became needed, the examinations made, and the ways in which data were summarized and presented, leading to the final conclusion.

A Case Study #1

1 Need

To determine whether the outfalls discharging raw sewage from a sewered population of 3820 persons in Villageville to River A between miles 20 and 18, causes a deterioration of water quality, thus constituting a hazard to the city water plant of Statesville with intake located at river mile 15.2 and the County Water Plant with the intake located at river mile 13.0.

2 Survey and procedures

A survey was established for the study of the reach of the river which included the pollution in the river above the alleged polluting city, pollution contributed from a tributary creek, the pollution contributed by Villageville and the effect on water quality at the water plant intakes. Samples were collected and examined every six hours for seven consecutive days. Bacteriological procedures were the Standard Methods multiple tube procedure by the confirmed test for the coliform group using three acceptable dilutions of 5/5/5; the test for coliforms of fecal origin; and the tentative plate count procedures for fecal streptococcal group and their confirmation by supporting biochemical tests.

3 Results

These data are presented in Table 3 and Figure 3.

4 Interpretation of the data

a The bacteriological data obtained at sample points located at river miles 24 to 21 inclusive, established the water in the river as being of relatively good quality, with total coliform density of approximately 600, 20 to 30 fecal coliforms and 10 to 16 fecal streptococci per 100 ml. This is a relatively high-quality raw-water river supply in this area.

b The Clear Creek tributary was an overflow from a lake with a coliform density of approximately 20 per 100 ml and both fecal coliforms and fecal streptococci were absent. In the absence of data on the cfs of Clear Creek and of River A, it is not possible to partition the bacterial densities at river mile 20 but it is evident that there is an apparent improvement in water quality due to the dilution factor.

c Between river mile 20 and 18, a marked increase in pollution occurred with 25,000, 10,000 and 4,000, respectively, being the densities of total coliform, fecal coliform and the fecal streptococcal groups. Periodic sampling of the sewer outfalls indicated the domestic waste from Villageville was the source of the bacterial densities.

d The bacterial data at river miles 16 to 10 inclusive demonstrated the poor quality of the water due to the pollution originating from the untreated wastes entering the river from Villageville.

e The presence of the fecal coliform group proves that this portion of the total coliform group originated from the gut of warm-blooded animals and is present in large quantities.

f The streptococcal group was proven by a series of biochemical reactions to be identical with the fecal streptococcal group found in the gut of warm-blooded animals and therefore confirmed the interpretation of the fecal coliform group.

g The presence of fecal pollution may be at any time and frequently is associated with enteric pathogenic bacteria, viral agents and parasitic organisms and by these associations, is a hazard to health.

h The presence of fecal pollution, as indicated by the data in Table 3, constitutes an unnecessary and remedial hazard and risk in the raw water supplies to the water plants.

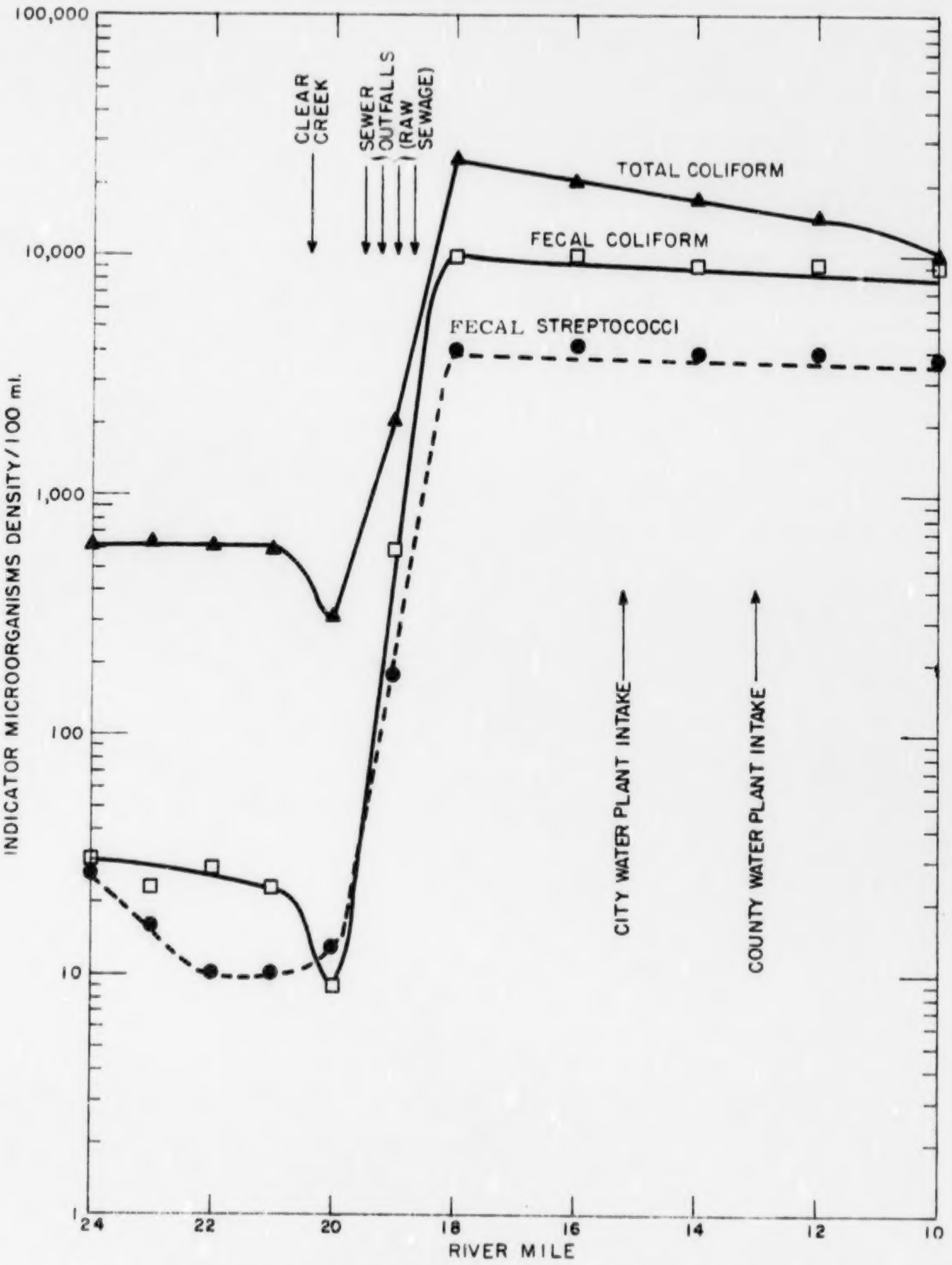
Table 3. INDICATOR MICROORGANISMS PER 100 ml IN RIVER A

River mile	Total Coliform	Fecal Coliform	Fecal Streptococci	Remarks
24	610	30	26	
23	620	23	16	
22	600	28	10	
21	590	23	10	
20	310	9	13	Clear Creek enters at river mile 20.4.
19	2,000	600	180	Four sewer outfalls between river miles 19.7 and 18.5 from Villageville.
18	25,000	10,000	4,000	
17				
16	20,000	10,000	4,200	
15				Statesville water intake at mile 15.2
14	17,000	9,000	3,900	
13				County water plant intake at mile 13.0
12	14,000	9,200	3,940	
11				
10	10,000	8,900	3,700	

EXPLANATION OF DATA

Bacterial densitities calculated as geometric mean value per 100 ml. Sample collected and examined every six hours for seven consecutive days.

Population (sewered) of Villageville, 3,820.
Consumers of water from city water plant, 70,000.
Consumers from county water plant, 205,000.
Velocity of river flow, 0.5 miles per hour.
Volume in cfs, information not available.
Report of sanitary survey by engineers: no known sources of pollution observed between river mile 24 and 10 except as noted under remarks.



A GRAPHIC PRESENTATION OF BACTERIOLOGICAL DATA

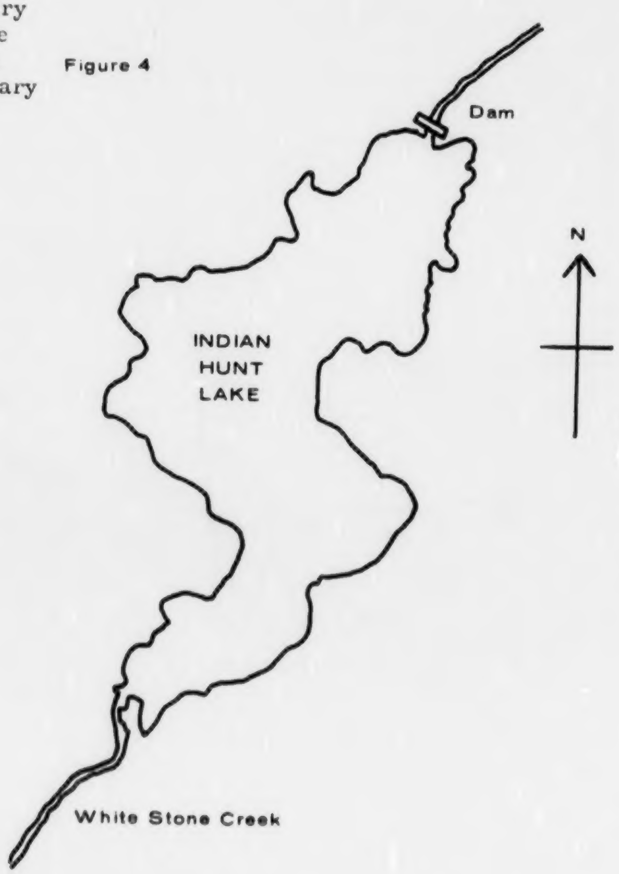
Figure 3

B Case Study #2

1 Need

A study was initiated at the request of a south western state to prepare a supplemental report on the bacteriological quality of a national wildlife refuge lake based upon state gathered data as well as an intensive study by the FWQA conducted during the same year. A massive localized fish kill and observed bacterial pollution indicator encroachments on the southern half of the lake precipitated an urgent need for this report. Figure 4 depicts this lake, Indian Hunt Lake, (here given a fictious name) and its major pollution tributary source. A closer view of this source in Figure 5 shows White Stone Creek as the major influence with its tributary Freehand Brook.

Figure 4



2 Survey and procedures

Indian Hunt Lake is a man made 2000 acre wildlife refuge and recreational area. Since there is a large population of waterfowl (exceeding 1, 025, 000 during resting and wintering periods) and contributing polluting sources from cattle feed lots, it was apparent that data would be required during dry periods and during rainfall and subsequent land runoff periods to determine the extent of this pollution effect. The necessity of this runoff data became even more manditory when it was ascertained that Freehand Brook was not a continual free-running tributary and this effect was only evident during periods of rainfall and the dry periods produced a dry bed and storage in Oxbow Lake. Stream flow determinations wer made and from 14 year averages peak flows were determined and in this manner maximum effects upon the Indian Hunt Lake could be more easily determined from current data.

Previous data for this study area was compiled by the state agency and consisted only of the total coliform indicator taken by the MPN method in monthly intervals. A four phase plan was established to accomplish the survey objectives:

a Acquire bacteriological data for all sample stations.

b Acquire additional bacteriological data and note the effect of recreational use on the bacteriological quality of Indian Hunt Lake.

c Evaluate the effect of rainfall on the receiving stream and Indian Hunt Lake.

d Data analysis and report preparation.

A mobile laboratory study was initiated and samples were analyzed by the membrane filter technique and the indicator organisms included the total coliforms, the fecal coliforms, and the fecal streptococci.

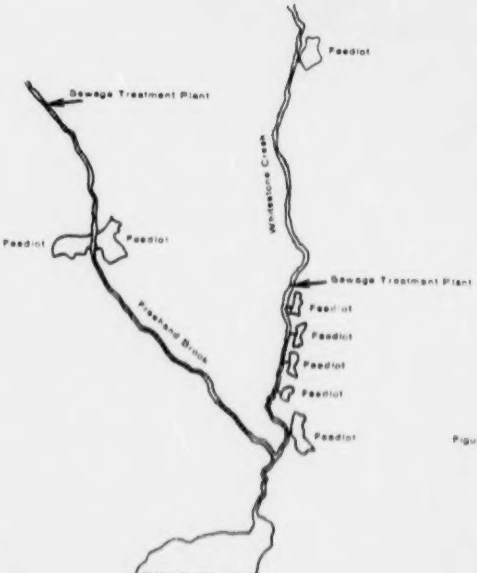
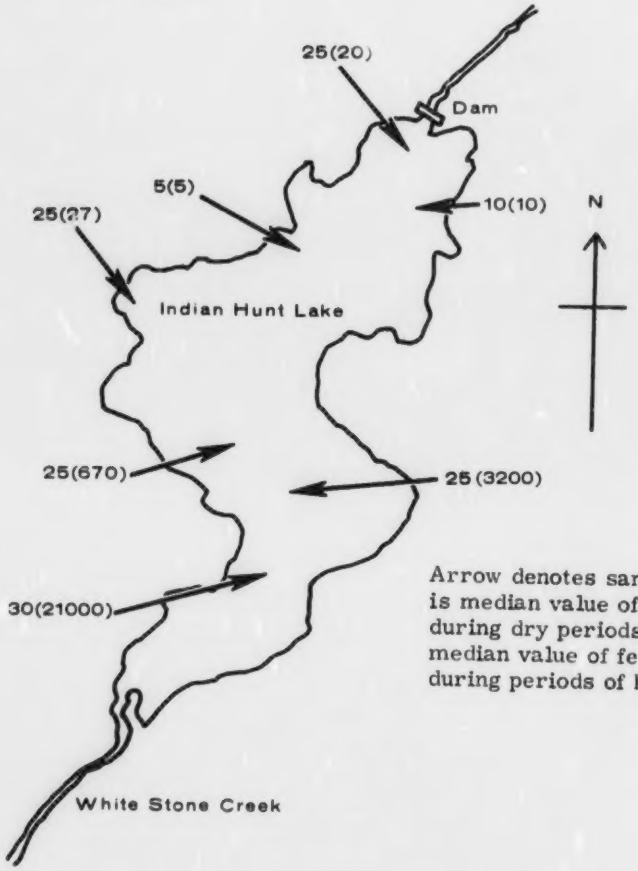


Figure 5

3 Results

After the acquisition of background data from the study area Indian Hunt Lake was sampled to ascertain the effect of recreational activities on the bacteriological quality during dry periods. Some of this data is reflected in Figure 6 which notes the fecal coliform median values per 100 ml for this period and the numbers in parenthesis gives gives the median values per 100 ml during periods of rainfall, heavy stormwater runoff, and subsequent encroachment of runoff bacteria into the lake.

Figure 6



Arrow denotes sampling point. First number is median value of fecal coliform per 100 ml during dry periods and second number is median value of fecal coliform per 100 ml during periods of heavy stormwater runoff.

4 Interpretation of data

Table 4 indicates the actual recommendations given by the final report and the column designated as Remarks gives the interpretations derived from the data.

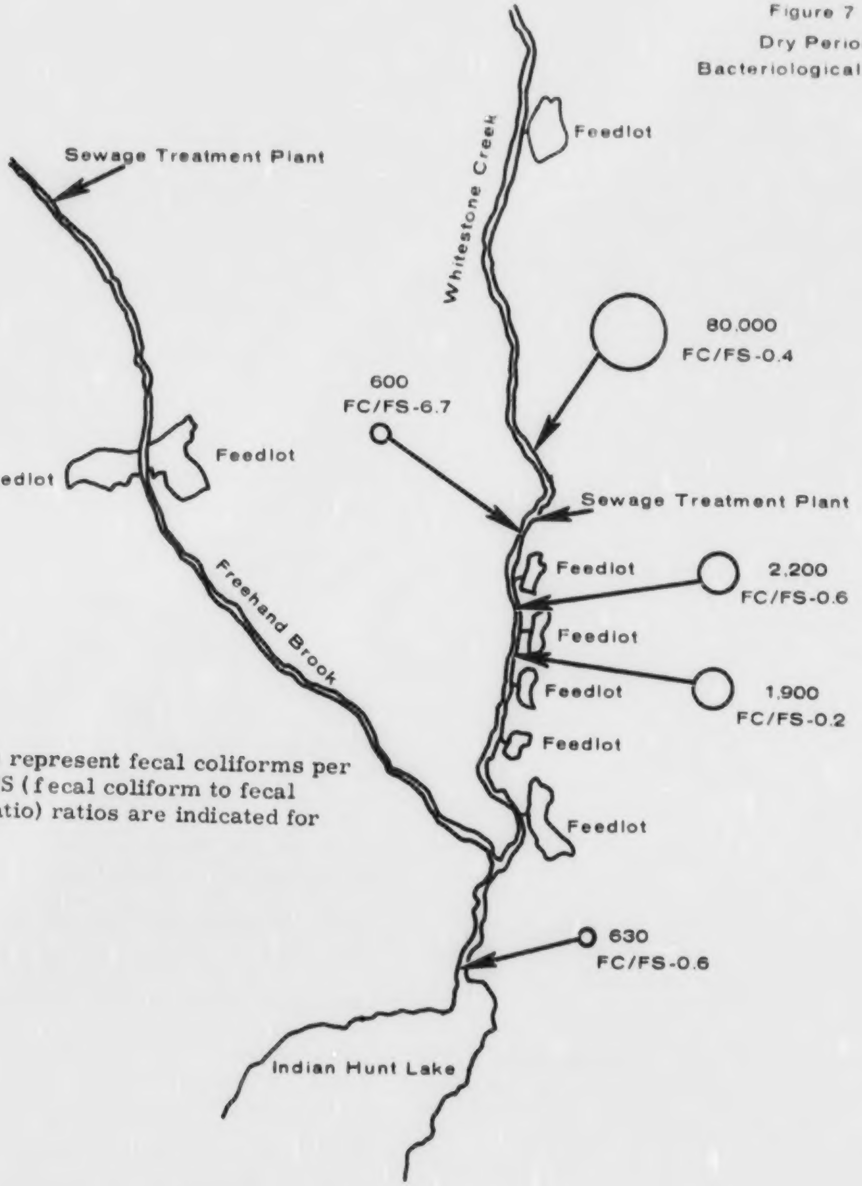
TABLE 4

Corrective Measures to More Adequately Control Discharges from Domestic Sewage and Cattle Feedlot Drainage into Indian Hunt Lake

Recommendations	Remarks
1. Development of diversion dykes around all cattle feedlots to channel drainage into waste stabilization ponds.	Influences of the cattle feedlot wastewater effluents are evident in Figure 7 and Figure 8 and this effect is pronounced during heavy stormwater overflows. Fecal coliform/ fecal streptococci ratios of less than 0.7 throughout most of the tributary and of White Stone Creek indicate the predominance of animal wastes other than human.
2. Existing sewage treatment facilities should be expanded to produce a better quality effluent with a BOD reduction goal of 85-90%. Post chlorination is desirable especially during the recreation season to further reduce pathogenic hazards during this period.	Enormous bacterial indicator contributions from the sewage treatment plant is evident in Figure 8 and the resultant FC/FS ratio confirms that the effluent is from domestic wastes (greater than 4).
3. Recreational restrictions will be necessary whenever intense rainfall occurs in a magnitude sufficient to produce an inflow of more than 450 acre feet of water from White Stone Creek.	These inflow measurements were made during measurements of flows occurring during periods of intense rainfall and noting the fecal coliform count intrusions to Indian Hunt Lake. With continual facility improvements this value could be revised upwards as effluent values exhibit lower bacterial parameter counts. Current standards of 200 fecal coliforms per 100 ml would determine when restrictions could be lifted for primary contact recreation.
4. Restrictions to be placed upon the horsepower and number of pleasure-boats in the area of inflow from White Stone Creek.	Since shallow areas exist in this lake it is necessary to include this provision to prevent and control the resuspension of indicator and pathogenic bacteria from the muds and deposits.
5. A buffer zone must be continually maintained and enforced between the wildlife refuge area and those areas designated for swimming, wading, and skiing.	Figure 9 depicts these areas and calendar dates. These designated buffer zones will lessen the potential health hazards from pathogens entrapped in the organic muck and lake water sediments. Pathogens gain entrance through inflow of White Stone Creek and to a more limited extent from the waterfowl.

A similar study was made of White Stone Creek and Freehand Brook and this is shown in Figure 7 and Figure 8. In both figures (Figure 7 - dry periods and Figure 8 - after heavy stormwater overflow) the circular areas indicate the fecal coliform values and also the FC/FS ratios (fecal coliform to fecal streptococci) are indicated for this sampling point.

Figure 7
Dry Period
Bacteriological Data

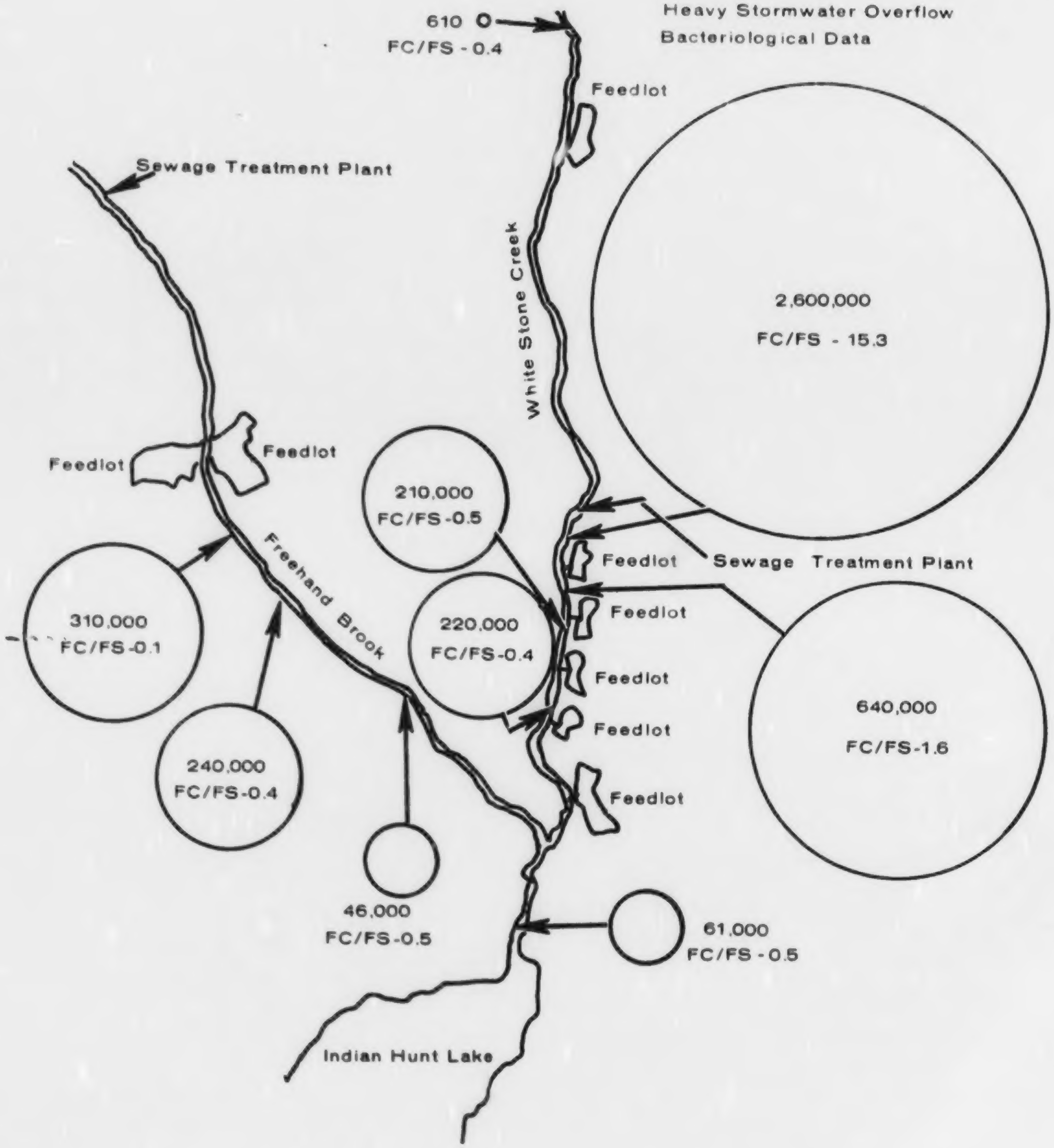

Sewage Treatment Plant
Whitestone Creek
Feedlot
80,000
FC/FS-0.4
600
FC/FS-6.7
Feedlot
Feedlot
Sewage Treatment Plant
Freehand Brook
Feedlot
2,200
FC/FS-0.6
Feedlot
Feedlot
1,900
FC/FS-0.2
Feedlot
Feedlot
630
FC/FS-0.6
Indian Hunt Lake

Circular areas represent fecal coliforms per 100 ml. FC/FS (fecal coliform to fecal streptococci ratio) ratios are indicated for each station.

20 14

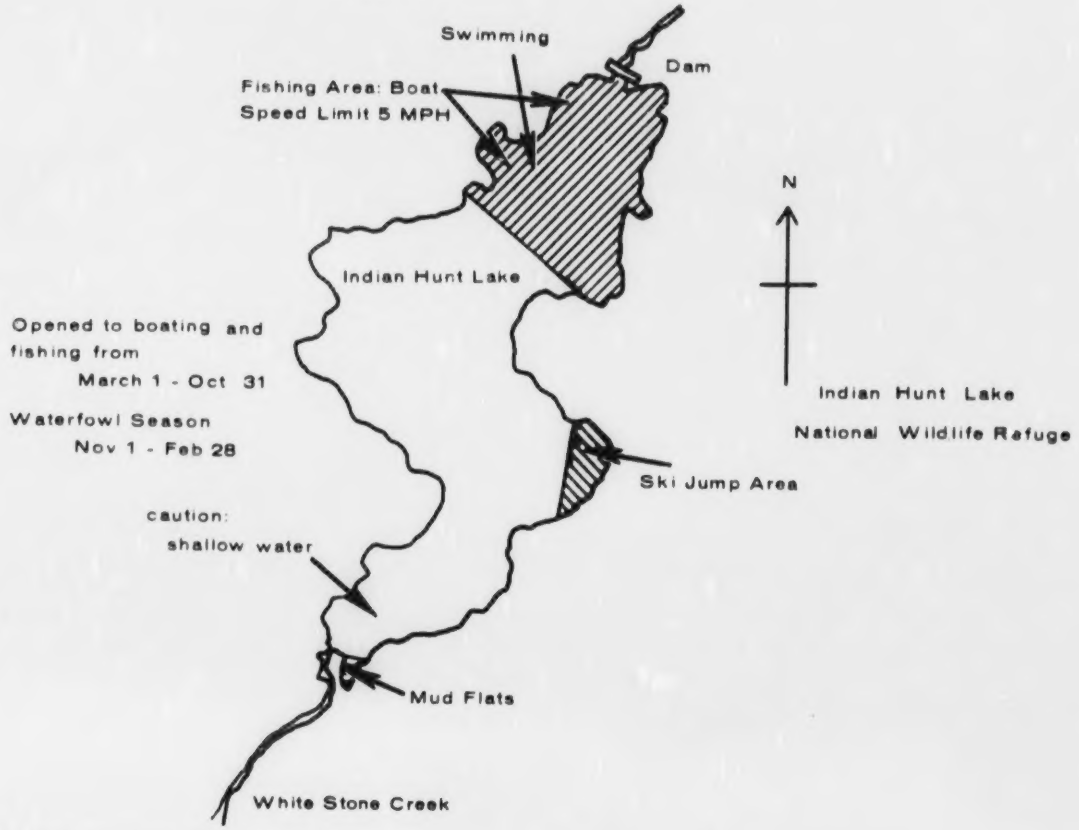
Figure 8

Heavy Stormwater Overflow Bacteriological Data


610
FC/FS - 0.4
Feedlot
Sewage Treatment Plant
White Stone Creek
2,600,000
FC/FS - 15.3
Feedlot
Feedlot
210,000
FC/FS -0.5
Freehand Brook
Feedlot
Sewage Treatment Plant
310,000
FC/FS -0.1
220,000
FC/FS -0.4
Feedlot
Feedlot
640,000
FC/FS -1.6
Feedlot
240,000
FC/FS -0.4
Feedlot
Feedlot
46,000
FC/FS -0.5
61,000
FC/FS - 0.5
Indian Hunt Lake

20.15

Figure 9


Swimming
Dam
Fishing Area: Boat Speed Limit 5 MPH
N
Indian Hunt Lake
Opened to boating and fishing from March 1 - Oct 31
Waterfowl Season Nov 1 - Feb 28
Indian Hunt Lake National Wildlife Refuge
Ski Jump Area
caution: shallow water
Mud Flats
White Stone Creek

ACKNOWLEDGEMENT:

In the preparation of this outline the author has made extensive use of material made available by Mr. Harold F. Clark (deceased) formerly bacteriological consultant to the Enforcement Branch, DWS & PC by Mr. F. W. Kittrell (retired) formerly Chief, Technical Advisory and Investigations Activities, FWQA and Mr. Edwin Geldreich, Chief Bacteriologist, Bureau of Water Hygiene, Environmental Protection Agency, Cincinnati, OH.

REFERENCES

1 Outline, this manual, titled "Bacteriological Indicators of Water Pollution" and related references.

2 Kittrell, F.W. and Furfari, S.A. Observations of Coliform Bacteria in Streams. Jour. Water Pollution Control Federation 35:1361-85. 1963.

This outline was prepared by H. L. Jeter, Director, and R. Russomanno, Microbiologist, National Training Center, WPO, EPA, Cincinnati, OH 45268.

Descriptors: Data Handling, Evaluation, Microbiology, Surveys, Water Pollution, Water Quality

WATER QUALITY SURVEYS

PREPARATION OF SURVEY REPORTS

I TYPE OF REPORT

The type of stream survey report to be prepared depends on two basic factors. These are the purpose and the audience for whom intended.

A The Purpose of the Report

1 A report of findings or basic data

2 A report of existing causes and effects together with an explanation of how and why.

3 An exposition of existing causes and effects and a projection of conditions that reasonably may occur due to natural variations in stream flow and temperature.

4 A purpose similar to 3 above plus a prediction of the effects of population growth and industrial change.

5 The same purpose as 4 above plus an estimate of the need to protect water uses, and cause reduction in waste loads.

B The Specific Audience for Whom the Report Is Prepared

1 For the record - no expository purpose

2 Other technical agencies with competencies in the same field

3 Other technical agencies in other fields

4 Public officials supporting or opposing the recommendations of the report

5 The general public

C Both in style and content the report should be adequate to serve as a basis for action to accomplish the recommended objectives.

II ORGANIZATION OF REPORT

A Title, Authors, Contents

B Acknowledgement of Aid and Assistance

1 Can be included in a letter of transmittal or submission

2 Can be incorporated in a preface or foreword

3 Should include names of persons and of corporations, public and private, who assisted or aided the survey

C Authority

1 Source of Authority

2 Date of authorization

D Report Summary

1 A brief summary of the report and its recommendations generally precedes the report proper and should include three topics:

a Summary of specific findings

b Conclusions drawn from findings

c Recommendations in general terms.

2 Brevity is essential but not at the expense of clarity.

3 A very brief but lucid description of the stream section involved should be included.

4 This will be the only part of the report read by many of its audience. Consequently it should be drafted with the utmost care.

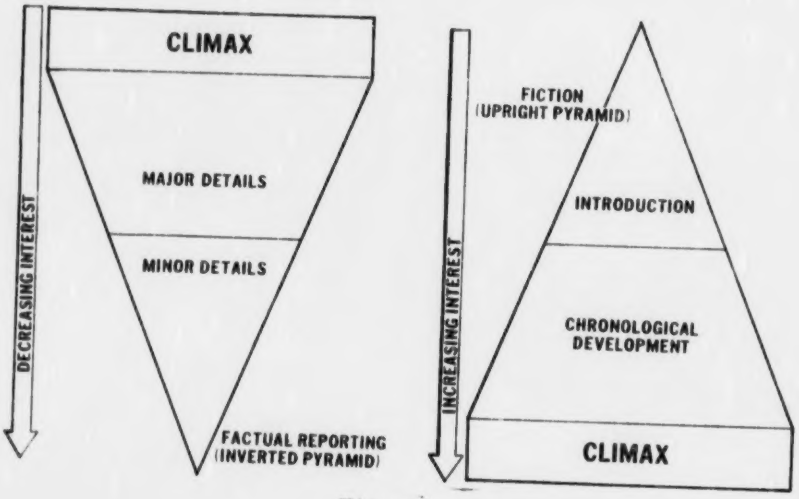

CLIMAX
MAJOR DETAILS
MINOR DETAILS
DECREASING INTEREST
FACTUAL REPORTING
(INVERTED PYRAMID)
FICTION
(UPRIGHT PYRAMID)
INTRODUCTION
CHRONOLOGICAL
DEVELOPMENT
INCREASING INTEREST
CLIMAX

Figure 1

5 Figure 1 illustrates the principles of written communication format. It is important that technical reports be presented in factual report arrangement and NOT in one similar to that of fiction.

6 Figure 2 shows how the various portions of a survey report relate to the generalized factual report.

7 Recommendations, although briefly stated in general terms, should be couched in positive, unexaggerated language.

8 Cost estimates of compliance with recommendations is helpful but not essential.

9 Both tangible and intangible benefits may be listed briefly under conclusions.

E INTRODUCTION

The body of the report should begin with a statement of the problem and a discussion of the reasons for and the location of the study.

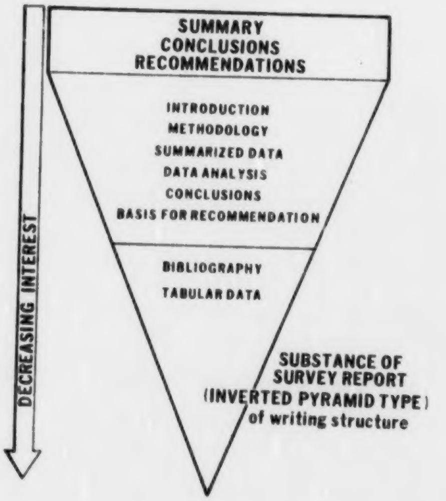

SUMMARY
CONCLUSIONS
RECOMMENDATIONS
INTRODUCTION
METHODOLOGY
SUMMARIZED DATA
DATA ANALYSIS
CONCLUSIONS
BASIS FOR RECOMMENDATION
BIBLIOGRAPHY
TABULAR DATA
DECREASING INTEREST
SUBSTANCE OF
SURVEY REPORT
(INVERTED PYRAMID TYPE)
of writing structure

Figure 2

1 Statement of the problem

a A description of the area, emphasizing pertinent features, should be included.

1) The inclusion of pertinent historical data is of value for audience orientation.

2) The relationship of this study to other current water resource studies.

3) Water use and economic data may be important.

b An area map is an absolute necessity.

1) Features included should be carefully selected and section of the stream involved should be emphasized.

2) Do not include unnecessary detail.

3) A general location map usually orients the reader to the area map.

2 Objectives of the Survey

a A statement of the purpose by listing the specific answers sought by the survey to address various aspects of the problem.

b The geographical and time scope of the objectives of the survey.

F Survey Methodology

A complete description of the methods of study employed is an important part of the record.

1 The time period of survey should be noted.

2 All sampling and gauging station locations should be identified by river mile.

3 Sampling and analytical methods

a Provide adequate description of all non-standard methods.

b An appendix for these descriptions may be required if they are lengthy.

4 Frequency of sampling

5 Description of laboratory types and locations

6 Hydrological methods employed for:

a Times of water travel

b Stream flow data

c Any waste flow measurements

G Survey Results

1 Sources of wastes

a Computed waste loads based on known contributing populations and industrial waste strength

b Results of sampling and gauging program

c Data summaries or displays suffice for text of report.

2 Stream data

a Summarized survey in the text

b Complete tabulations including time of collection and averages in appendix

3 Hydrological data

a Usually tabulated with analytical results both in the text and in appendices

b Time of water travel curve or curves

c Stream flow frequency charts

d Pertinent groundwater data

4 Aesthetic considerations are of real importance.

H Analysis and Interpretation of Data

1 Three fundamental procedures are required:

a Comparison of survey results and appropriate water quality criteria

b Projection of survey data to provide for comparison of stream conditions with water quality criteria under more adverse conditions

c Estimates of permissible wastes loads under present and future conditions.

2 Since this topic is the subject of other outlines it will not be further developed here.

3 A description of the methods of analysis and interpretation belong in the report.

a Necessary assumptions should be stated.

b All statistical methods used should be identified.

4 Results of analysis and interpretation are best presented in chart form insofar as possible, to support discussion and interpretation rationale.

I Conclusions

1 Focus of attention, much of it critical, is on this section of the report.

2 Clearness, conciseness, and positiveness are essential.

3 This section indicates reasoning that leads from findings to conclusions.

J Recommendations

1 This is the crux of the report and should answer the question, "What needs to be done to resolve problems delineated in the report?"

2 Cost estimates are highly desirable, if possible, and appropriate.

K Bibliography

1 Useful to the student and perhaps to future workers

2 Entails additional time and effort in assembling references in proper form

III TECHNIQUES AND TOOLS

A Prepare a Detailed Outline

1 This is an important step if the coverage of the report is to be completed and its arrangement logical. It is also a timesaver if properly done.

2 Topics to be included in the outline will become apparent from the foregoing discussion of report content.

B Words are Your Tools

1 Keep dictionary, thesaurus, and glossary available.

2 Define any vague terms or abbreviations.

3 Control superlatives and slang.

4 Avoid emphatics such as "it is to be noted" or "it is a well-known fact".

5 Limit intensive expressions, such as "extremely" or "undoubtedly."

6 Use active verbs when possible.

C Regularly review the report organization and development with a colleague.

This outline was prepared by Staff Members, National Training Center, EPA, WPO, Cincinnati, OH 45268

Descriptors: Reports, Surveys, Technical Writing, Water Quality

THE AQUATIC ENVIRONMENT

Part 1: The Nature and Behavior of Water

I INTRODUCTION

The earth is physically divisible into the lithosphere or land masses, and the hydrosphere which includes the oceans, lakes, streams, and subterranean waters.

A Upon the hydrospere are based a number of sciences which represent different approaches. Hydrology is the general science of water itself with its various special fields such as hydrography, hydraulics, etc. These in turn merge into physical chemistry and chemistry.

B Limnology and oceanography combine aspects of all of these, and deal not only with the physical liquid water and its various naturally occurring solutions and forms, but also with living organisms and the infinite interactions that occur between them and their environment.

C Water quality management, including pollution control, thus looks to all branches of aquatic science in efforts to coordinate and improve man's relationship with his aquatic environment.

II SOME FACTS ABOUT WATER

A Water is the only abundant liquid on our planet. It has many properties most unusual for liquids, upon which depend most of the familiar aspects of the world about us as we know it. (See Table 1)

TABLE 1

UNIQUE PROPERTIES OF WATER

Property	Significance
Highest heat capacity (specific heat) of any solid or liquid (except NH_3)	Stabilizes temperatures of organisms and geographical regions
Highest latent heat of fusion (except NH_3)	Thermostatic effect at freezing point
Highest heat of evaporation of any substance	Important in heat and water transfer of atmosphere
The only substance that has its maximum density as a liquid (4°C)	Fresh and brackish waters have maximum density above freezing point. This is important in vertical circulation pattern in lakes.
Highest surface tension of any liquid	Controls surface and drop phenomena, important in cellular physiology
Dissolves more substances in greater quantity than any other liquid	Makes complex biological system possible. Important for transportation of materials in solution.
Pure water has the highest di-electric constant of any liquid	Leads to high dissociation of inorganic substances in solution
Very little electrolytic dissociation	Neutral, yet contains both H^+ and OH^- ions
Relatively transparent	Absorbs much energy in infra red and ultra violet ranges, but little in visible range. Hence "colorless"

B Physical Factors of Significance

1 Water substance

Water is not simply "H_2O" but in reality is a mixture of some 33 different substances involving three isotopes each of hydrogen and oxygen (ordinary hydrogen H^1, deuterium H^2, and tritium H^3; ordinary oxygen O^{16}, oxygen 17, and oxygen 18) plus 15 known types of ions. The molecules of a water mass tend to associate themselves as polymers rather than to remain as discrete units. (See Figure 1)

SUBSTANCE OF PURE WATER

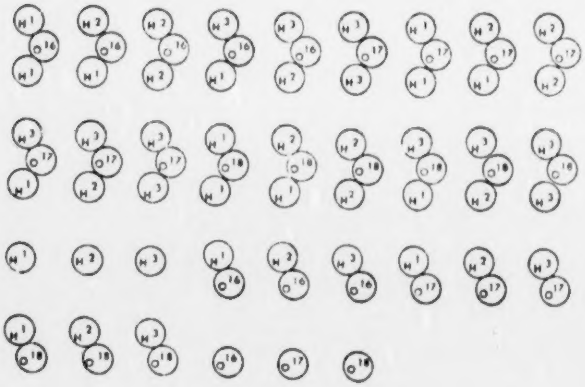


Figure 1

2 Density

a Temperature and density: Ice. Water is the only known substance in which the solid state will float on the liquid state. (See Table 2)

This ensures that ice usually forms on top of a body of water and tends to insulate the remaining water mass from further loss of heat. Did ice sink, there could be little or no carryover of aquatic life from season to season in the higher latitudes. Frazil or needle ice forms colloidally at a few thousandths of a degree below 0° C. It is adhesive and may build up on submerged objects as "anchor ice", but it is still typical ice (ice I).

TABLE 2

EFFECTS OF TEMPERATURE ON DENSITY OF PURE WATER AND ICE*

Temperature (°C)	Density	
	Water	Ice**
-10	.99815	.9397
- 8	.99869	.9360
- 6	.99912	.9020
- 4	.99945	.9277
- 2	.99970	.9229
0	.99987----	.9168
2	.99997	
4	1.00000	
6	.99997	
8	.99988	
10	.99973	
20	.99823	
40	.99225	
60	.98324	
80	.97183	
100	.95838	

* Tabular values for density, etc., represent estimates by various workers rather than absolute values, due to the variability of water.

** Regular ice is known as "ice I". Four or more other "forms" of ice are known to exist (ice II, ice III, etc.), having densities at 1 atm. pressure ranging from 1.1595 to 1.67. These are of extremely restricted occurrence and may be ignored in most routine operations.

1) Seasonal increase in solar radiation annually warms surface waters in summer while other factors result in winter cooling. The density differences resulting establish two classic layers: the epilimnion or surface layer, and the hypolimnion or lower layer, and in between is the thermocline or shear-plane.

2) While for certain theoretical purposes a "thermocline" is defined as a zone in which the temperature changes one degree centigrade for each meter of depth, in practice, any transitional layer between two relatively stable masses of water of different temperatures may be regarded as a thermocline.

3) Obviously the greater the temperature differences between epilimnion and hypolimnion and the sharper the gradient in the thermocline, the more stable will the situation be.

4) From information given above, it should be evident that while the temperature of the hypolimnion rarely drops much below 4° C, the epilimnion may range from 0° C upward.

5) When epilimnion and hypolimnion achieve the same temperature, stratification no longer exists. The entire body of water behaves hydrologically as a unit, and tends to assume uniform chemical and physical characteristics. Even a light breeze may then cause the entire body of water to circulate. Such events are called overturns, and usually result in water quality changes of considerable physical, chemical, and biological significance. Mineral-rich water from the hypolimnion, for example, is mixed with oxygenated water from the epilimnion. This usually triggers a sudden growth or "bloom" of plankton organisms.

6) When stratification is present, however, each layer behaves relatively independently, and significant quality differences may develop.

7) Thermal stratification as described above has no reference to the size of the water mass; it is found in oceans and puddles.

b The relative densities of the various isotopes of water influence its molecular composition. For example, the lighter O_{16} tends to go off first in the process of evaporation, leading to the relative enrichment of air by O_{16} and the enrichment of water by O_{17} and O_{18}. This can lead to a measurably higher O_{18} content in warmer climates. Also, the temperature of water in past geologic ages can be closely estimated from the ratio of O_{18} in the carbonate of mollusc shells.

c Dissolved and/or suspended solids may also affect the density of natural water masses (see Table 3)

TABLE 3
EFFECTS OF DISSOLVED SOLIDS ON DENSITY

Dissolved Solids (Grams per liter)	Density (at 4° C)
0	1.00000
1	1.00085
2	1.00169
3	1.00251
10	1.00818
35 (mean for sea water)	1.02822

d Types of density stratification

1) Density differences produce stratification which may be permanent, transient, or seasonal.

2) Permanent stratification exists for example where there is a heavy mass of brine in the deeper areas of a basin which does not respond to seasonal or other changing conditions.

3) Transient stratification may occur with the recurrent influx of tidal water in an estuary for example, or the occasional influx of cold muddy water into a deep lake or reservoir.

4) Seasonal stratification is typically thermal in nature, and involves the annual establishment of the epilimnion, hypolimnion, and thermocline as described above.

5) Density stratification is not limited to two-layered systems; three, four, or even more layers may be encountered in larger bodies of water.

e A "plunge line" (sometimes called "thermal line") may develop at the mouth of a stream. Heavier water flowing into a lake or reservoir plunges below the lighter water mass of the epiliminium to flow along at a lower level. Such a line is usually marked by an accumulation of floating debris.

f Stratification may be modified or entirely suppressed in some cases when deemed expedient, by means of a simple air lift.

3 The viscosity of water is greater at lower temperatures (see Table 4). This is important not only in situations involving the control of flowing water as in a sand filter, but also since overcoming resistance to flow generates heat, it is significant in the heating of water by internal friction from wave and current action. Living organisms more easily support themselves in the more viscous (and also denser) cold waters of the arctic than in the less viscous warm waters of the tropics. (See Table 4).

TABLE 4

VISCOSITY OF WATER (In millipoises at 1 atm)

Temp. °C	Dissolved solids in g/L			
	0	5	10	30
-10	26.0	----	----	----
- 5	21.4	----	----	----
0	17.94	18.1	18.24	18.7
5	15.19	15.3	15.5	16.0
10	13.10	13.2	13.4	13.8
30	8.00	8.1	8.2	8.6
100	2.84	----	----	----

4 Surface tension has biological as well as physical significance. Organisms whose body surfaces cannot be wet by water can either ride on the surface film or in some instances may be "trapped" on the surface film and be unable to re-enter the water.

5 Heat or energy

Incident solar radiation is the prime source of energy for virtually all organic and most inorganic processes on earth. For the earth as a whole, the total amount (of energy) received annually must exactly balance that lost by reflection and radiation into space if climatic and related conditions are to remain relatively constant over geologic time.

a For a given body of water, immediate sources of energy include in addition to solar irradiation: terrestrial heat, transformation of kinetic energy (wave and current action) to heat, chemical and biochemical reactions, convection from the atmosphere, and condensation of water vapor.

b The proportion of light reflected depends on the angle of incidence, the temperature, color, and other qualities of the water; and the presence or absence of films of lighter liquids such as oil. In general, as the depth increases arithmetically, the light tends to decrease geometrically. Blues, greens, and yellows tend to penetrate most deeply while ultra violet, violets, and orange-reds are most quickly absorbed. On the order of 90% of the total illumination which penetrates the surface film is absorbed in the first 10 meters of even the clearest water, thus tending to warm the upper layers.

6 Water movements

a Waves or rhythmic movement

1) The best known are traveling waves caused by wind. These are effective only against objects near the surface. They have little effect on the movement of large masses of water.

2) Seiches

Standing waves or seiches occur in lakes, estuaries, and other enclosed bodies of water, but are seldom large enough to be observed. An "internal wave or seich" is an oscillation in a submersed mass of water such as a hypolimnion, accompanied by compensating oscillation in the overlying water so that no significant change in surface level is detected. Shifts in submerged water masses of this type can have severe effects on the biota and also on human water uses where withdrawals are confined to a given depth. Descriptions and analyses of many other types and sub-types of waves and wave-like movements may be found in the literature.

b Tides

1) Tides are the longest waves known, and are responsible for the once or twice a day rythmic rise and fall of the ocean level on most shores around the world.

2) While part and parcel of the same phenomenon, it is often convenient to refer to the rise and fall of the water level as "tide," and to the resulting currents as "tidal currents."

3) Tides are basically caused by the attraction of the sun and moon on water masses, large and small; however, it is only in the oceans and possibly certain of the larger lakes that true tidal action has been demonstrated. The patterns of tidal action are enormously complicated by local topography, interaction with seiches, and other factors. The literature on tides is very large.

c Currents (except tidal currents) are steady arythmic water movements which have had major study only in oceanography although they are most often observed in rivers and streams. They are primarily concerned with the translocation of water masses. They may be generated internally by virtue of density changes, or externally by wind or terrestrial topography. Turbulence phenomena or eddy currents are largely responsible for lateral mixing in a current. These are of far more importance in the economy of a body of water than mere laminar flow.

d Coriolis force is a result of interaction between the rotation of the earth, and the movement of masses or bodies on the earth. The net result is a slight tendency for moving objects to veer to the right in the northern hemisphere, and to the left in the southern hemisphere. While the result in fresh waters is usually negligible, it may be considerable in marine waters. For example, other factors permitting, there is a tendency in estuaries for fresh waters to move toward the ocean faster along the right bank, while salt tidal waters tend to intrude farther inland along the left bank. Effects are even more dramatic in the open oceans.

e Langmuir circulation (or L. spirals) is the interlocking rotation of somewhat cylindrical masses of surface water under the influence of wind action. The axes of the cylinders are parallel to the direction of the wind.

To somewhat oversimplify the concept, a series of adjoining cells might be thought of as chains of interlocking gears in which at every other contact the teeth are rising while at the alternate contacts, they are sinking (Figure 2).

The result is elongated masses of water rising or sinking together. This produces the familiar "wind rows" of foam, flotsam and jetsam, or plankton often seen streaking windblown lakes or oceans. Certain zoo-plankton struggling to maintain a position near the surface tend to collect in the down current between two Langmuir cells, causing such an area to be called the "red dance", while the clear upwelling water between is the "blue dance".

This phenomenon may be important in water or plankton sampling on a windy day.

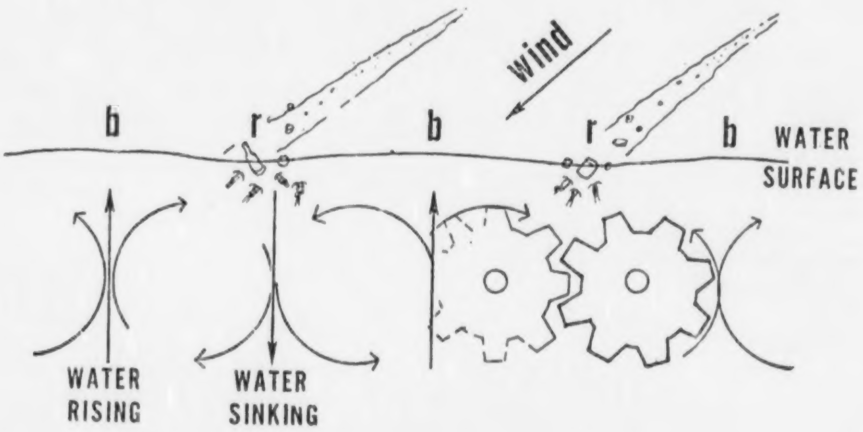


Figure 2. Langmuire Spirals
b. Blue dance, water rising. r. Red dance, water sinking, floating or swimming objects concentrated.

6 The pH of pure water has been determined between 5.7 and 7.01 by various workers. The latter value is most widely accepted at the present time. Natural waters of course vary widely according to circumstances.

C The elements of hydrology mentioned above represent a selection of some of the more conspicuous physical factors involved in working with water quality. Other items not specifically mentioned include: molecular structure of waters, interaction of water and radiation, internal pressure, acoustical characteristics, pressure-volume-temperature relationships, refractivity, luminescence, color, dielectrical characteristics and phenomena, solubility, action and interactions of gases, liquids and solids, water vapor, phenomena of hydrostatics and hydrodynamics in general.

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Descriptors:
Aquatic Environment, Estuarine Environment, Lentic Environment, Lotic Environment, Currents, Marshes, Limnology, Water Properties

THE AQUATIC ENVIRONMENT

Part 2: The Aquatic Environment as an Ecosystem

I INTRODUCTION

Part 1 introduced the lithosphere and the hydrosphere. Part 2 will deal with certain general aspects of the biosphere, or the sphere of life on this earth, which photographs from space have shown is a finite globe in infinite space.

This is the habitat of man and the other organisms. His relationships with the aquatic biosphere are our common concern.

II THE BIOLOGICAL NATURE OF THE WORLD WE LIVE IN

A We can only imagine what this world must have been like before there was life.

B The world as we know it is largely shaped by the forces of life.

1 Primitive forms of life created organic matter and established soil.

2 Plants cover the lands and enormously influence the forces of erosion.

3 The nature and rate of erosion affect the redistribution of materials (and mass) on the surface of the earth (topographic changes).

4 Organisms tie up vast quantities of certain chemicals, such as carbon and oxygen.

5 Respiration of plants and animals releases carbon dioxide to the atmosphere in influential quantities.

6 CO_2 affects the heat transmission of the atmosphere.

C Organisms respond to and in turn affect their environment. Man is one of the most influential.

III ECOLOGY IS THE STUDY OF THE INTERRELATIONSHIPS BETWEEN ORGANISMS, AND BETWEEN ORGANISMS AND THEIR ENVIRONMENT.

A The ecosystem is the basic functional unit of ecology. Any area of nature that includes living organisms and nonliving substances interacting to produce an exchange of materials between the living and nonliving parts constitutes an ecosystem. (Odum, 1959)

1 From a structural standpoint, it is convenient to recognize four constituents as composing an ecosystem (Figure 1).

a Abiotic NUTRIENT MINERALS which are the physical stuff of which living protoplasm will be synthesized.

b Autotrophic (self-nourishing) or PRODUCER organisms. These are largely the green plants (holophytes), but other minor groups must also be included (See Figure 2). They assimilate the nutrient minerals, by the use of considerable energy, and combine them into living organic substance.

c Heterotrophic (other-nourishing) CONSUMERS (holozoic), are chiefly the animals. They ingest (or eat) and digest organic matter, releasing considerable energy in the process.

d Heterotrophic REDUCERS are chiefly bacteria and fungi that return complex organic compounds back to the original abiotic mineral condition, thereby releasing the remaining chemical energy.

2 From a functional standpoint, an ecosystem has two parts (Figure 2)

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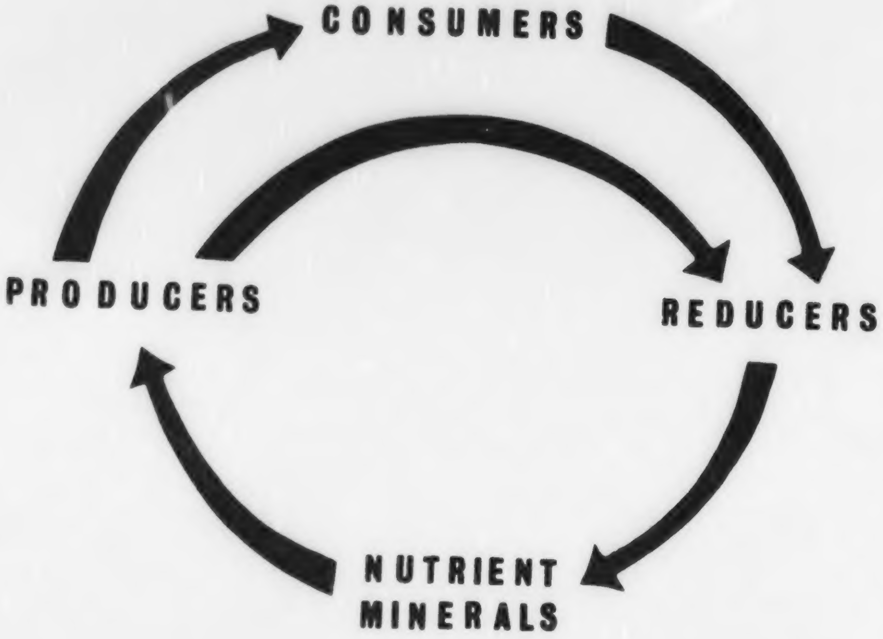


FIGURE 1

a The autotrophic or producer organisms, which construct organic substance.

b The heterotrophic or consumer and reducer organisms which destroy organic substance.

3 Unless the autotrophic and heterotrophic phases of the cycle approximate a dynamic equilibrium, the ecosystem and the environment will change.

B Each of these groups includes simple, single-celled representatives, persisting at lower levels on the evolutionary stems of the higher organisms. (Figure 2)

1 These groups span the gaps between the higher kingdoms with a multitude of transitional forms. They are collectively called the PROTISTA.

2 Within the protista, two principal subgroups can be defined on the basis of relative complexity of structure.

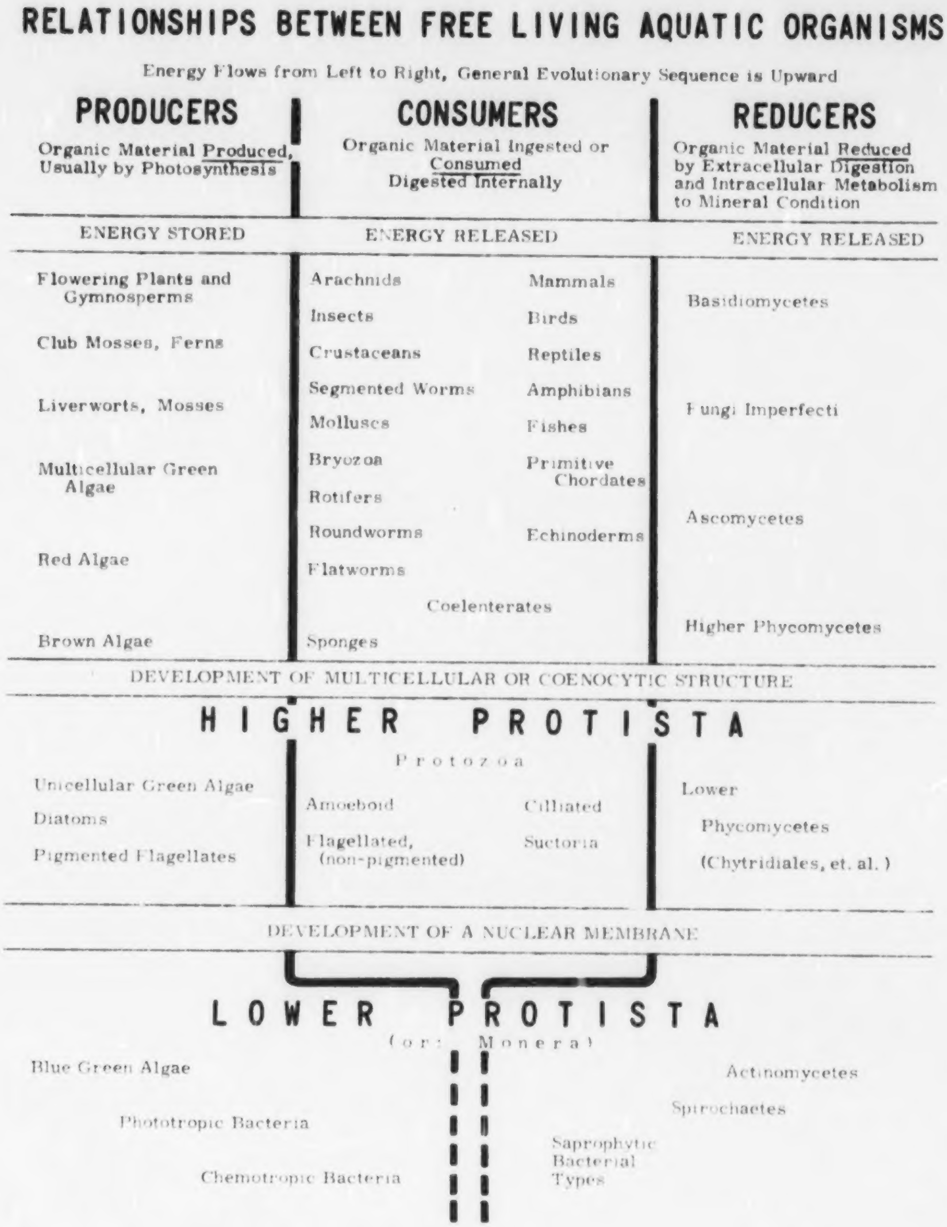
a The bacteria and blue-green algae, lacking a nuclear membrane may be considered as the lower protista (or Monera).

b The single-celled algae and protozoa are best referred to as the Higher Protista.

C Distributed throughout these groups will be found most of the traditional "phyla" of classic biology.

IV FUNCTIONING OF THE ECOSYSTEM

A A food chain is the transfer of food energy from plants through a series of organisms with repeated eating and being eaten. Food chains are not isolated sequences but are interconnected.



RELATIONSHIPS BETWEEN FREE LIVING AQUATIC ORGANISMS

Energy Flows from Left to Right, General Evolutionary Sequence is Upward

PRODUCERS	CONSUMERS		REDUCERS
Organic Material Produced, Usually by Photosynthesis	Organic Material Ingested or Consumed Digested Internally		Organic Material Reduced by Extracellular Digestion and Intracellular Metabolism to Mineral Condition
ENERGY STORED	ENERGY RELEASED		ENERGY RELEASED
Flowering Plants and Gymnosperms	Arachnids	Mammals	Basidiomycetes
	Insects	Birds	
Club Mosses, Ferns	Crustaceans	Reptiles	
Liverworts, Mosses	Segmented Worms	Amphibians	Fungi Imperfecti
	Molluscs	Fishes	
Multicellular Green Algae	Bryozoa	Primitive Chordates	
	Rotifers		Ascomycetes
Red Algae	Roundworms	Echinoderms	
	Flatworms		
	Coelenterates		
Brown Algae	Sponges		Higher Phycomycetes
DEVELOPMENT OF MULTICELLULAR OR COENOCYTIC STRUCTURE			
HIGHER PROTISTA			
	Protozoa		
Unicellular Green Algae	Amoeboid	Ciliated	Lower Phycomycetes
Diatoms	Flagellated, (non-pigmented)	Suctoria	(Chytridiales, et. al.)
Pigmented Flagellates			
DEVELOPMENT OF A NUCLEAR MEMBRANE			

LOWER PROTISTA

(or: Monera)

Blue Green Algae

Phototropic Bacteria

Chemotropic Bacteria

Actinomycetes

Spirochaetes

Saprophytic Bacterial Types

FIGURE 2

BI ECO pl. 2a. 1. 69

B **A food web** is the interlocking pattern of food chains in an ecosystem. (Figures 3, 4) In complex natural communities, organisms whose food is obtained by the same number of steps are said to belong to the same trophic (feeding) level.

C **Trophic Levels**

1 First - Green plants (producers) (Figure 5) fix biochemical energy and synthesize basic organic substances. This is "primary production".

2 Second - Plant eating animals (herbivores) depend on the producer organisms for food.

3 Third - Primary carnivores, animals which feed on herbivores.

4 Fourth - Secondary carnivores feed on primary carnivores.

5 Last - Ultimate carnivores are the last or ultimate level of consumers.

D **Total Assimilation**

The amount of energy which flows through a trophic level is distributed between the production of biomass (living substance), and the demands of respiration (internal energy use by living organisms) in a ratio of approximately 1:10.

E **Trophic Structure** of the Ecosystem

The interaction of the food chain phenomena (with energy loss at each transfer) results in various communities having definite trophic structure or energy levels. Trophic structure may be measured and described either in terms of the standing crop per unit area or in terms of energy fixed per unit area per unit time at successive trophic levels. Trophic structure and function can be shown graphically by means of ecological pyramids (Figure 5).

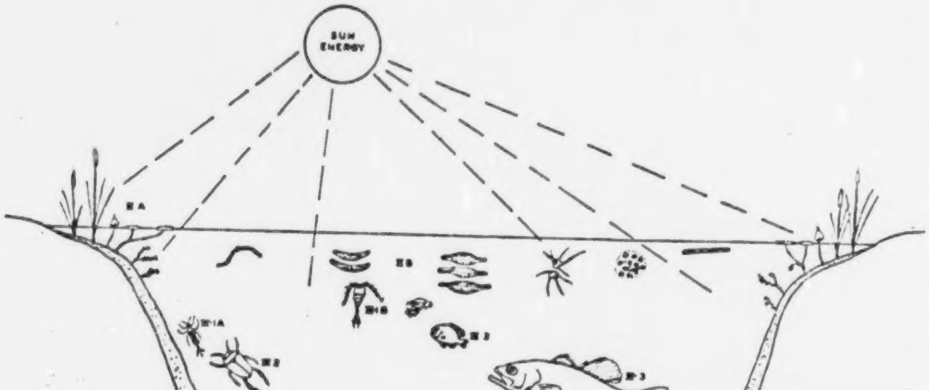
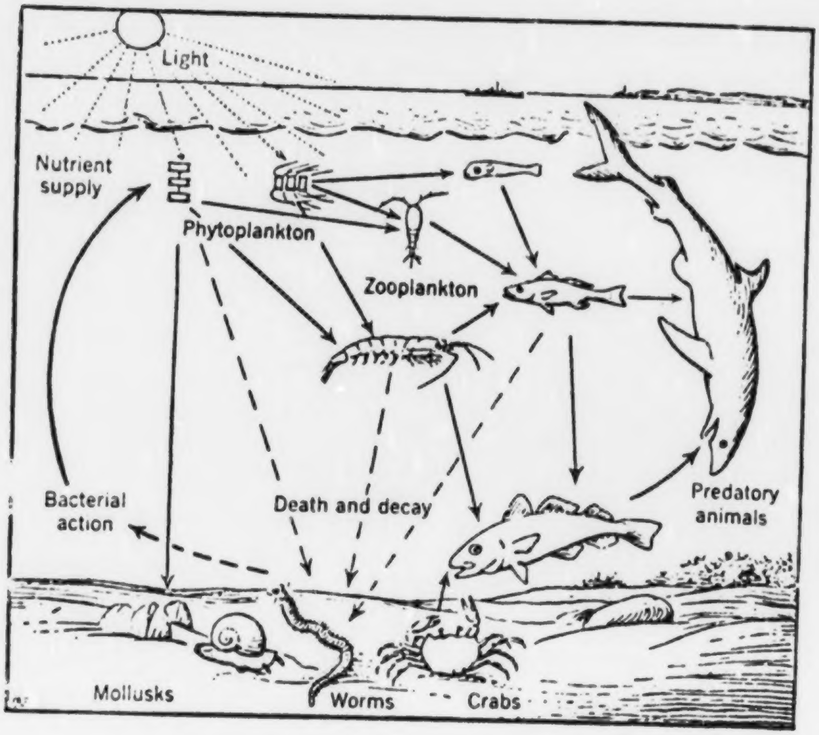


Figure 3. Diagram of the pond ecosystem. Basic units are as follows: I, abiotic substances–basic inorganic and organic compounds; IIA, producers–rooted vegetation; IIB, producers–phytoplankton; III-1A, primary consumers (herbivores)–bottom forms; III-1B, primary consumers (herbivores)–zooplankton; III-2, secondary consumers (carnivores); III-3, tertiary consumers (secondary carnivores); IV, decomposers–bacteria and fungi of decay.


Light
Nutrient supply
Phytoplankton
Zooplankton
Bacterial action
Death and decay
Predatory animals
Mollusks
Worms
Crabs

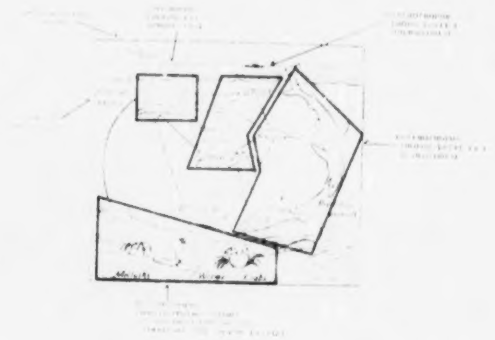


Figure 4. A MARINE ECOSYSTEM (After Clark, 1954 and Patten, 1966)

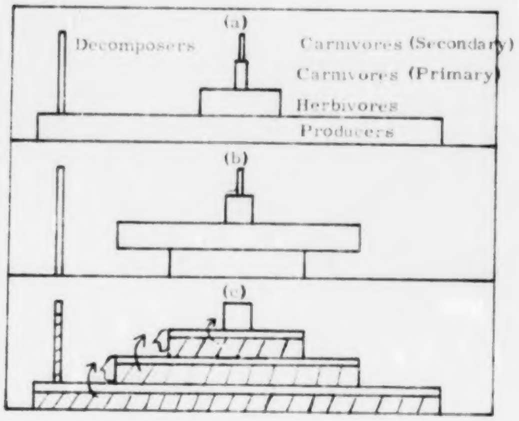


Figure 5. HYPOTHETICAL PYRAMIDS of (a) Numbers of individuals, (b) Biomass, and (c) Energy (Shading Indicates Energy Loss).

V BIOTIC COMMUNITIES

A Plankton are the macroscopic and microscopic animals, plants, bacteria, etc., floating free in the open water. Many clog filters, cause tastes, odors, and other troubles in water supplies. Eggs and larvae of larger forms are often present.

1 Phytoplankton are plant-like. These are the dominant producers of the waters, fresh and salt, "the grass of the seas".

2 Zooplankton are animal-like. Includes many different animal types, range in size from minute protozoa to gigantic marine jellyfishes.

B Periphyton (or Aufwuchs) - The communities of microscopic organisms associated with submerged surfaces of any type or depth. Includes bacteria, algae, protozoa, and other microscopic animals, and often the young or embryonic stages of algae and other organisms that normally grow up to become a part of the benthos (see below). Many planktonic types will also adhere to surfaces as periphyton, and some typical periphyton may break off and be collected as plankters.

C Benthos are the plants and animals living on, in, or closely associated with the bottom. They include plants and invertebrates.

D Nekton are the community of strong aggressive swimmers of the open waters, often called pellagic. Certain fishes, whales, and invertebrates such as shrimps and squids are included here.

E The marsh community is based on larger "higher" plants, floating and emergent. Both marine and freshwater marshes are areas of enormous biological production. Collectively known as "wetlands", they bridge the gap between the waters and the dry lands.

VI PRODUCTIVITY

A The biological resultant of all physical and chemical factors in the quantity of life that may actually be present. The ability to produce this "biomass" is often referred to as the "productivity" of a body of water. This is neither good nor bad per se. A water of low productivity is a "poor" water biologically, and also a relatively "pure" or "clean" water; hence desirable as a water supply or a bathing beach. A productive water on the other hand may be a nuisance to man or highly desirable. It is a nuisance if foul odors and/or weed-chocked waterways result, it is desirable if bumper crops of bass, catfish, or oysters are produced. Open oceans have a low level of productivity in general.

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Descriptors:
Aquatic Environment, Estuarine Environment, Lentic Environment, Lotic Environment, Currents, Marshes, Limnology, Water Properties

THE AQUATIC ENVIRONMENT

Part 3. The Freshwater Environment

I INTRODUCTION

The freshwater environment as considered herein refers to those inland waters not detectably diluted by ocean waters, although the lower portions of rivers are subject to certain tidal flow effects.

Certain atypical inland waters such as saline or alkaline lakes, springs, etc., are not treated, as the main objective here in typical inland water.

All waters have certain basic biological cycles and types of interactions most of which have already been presented, hence this outline will concentrate on aspects essentially peculiar to fresh inland waters.

II PRESENT WATER QUALITY AS A FUNCTION OF THE EVOLUTION OF FRESH WATERS

A The history of a body of water determines its present condition. Natural waters have evolved in the course of geologic time into what we know today.

B Streams

In the course of their evolution, streams in general pass through four stages of development which may be called: birth, youth, maturity, and old age.

These terms or conditions may be employed or considered in two contexts: temporal, or spatial. In terms of geologic time, a given point in a stream may pass through each of the stages described below or: at any given time, these various stages of development can be loosely identified in successive reaches of a stream traveling from its headwaters to base level in ocean or major lake.

1 Establishment or birth. This might be a "dry run" or headwater stream-bed, before it had eroded down to the level of ground water.

During periods of run-off after a rain or snow-melt, such a gulley would have a flow of water which might range from torrential to a mere trickle. Erosion may proceed rapidly as there is no permanent aquatic flora or fauna to stabilize streambed materials. On the other hand, terrestrial grass or forest growth may retard erosion. When the run-off has passed, however, the "streambed" is dry.

2 Youthful streams. When the streambed is eroded below the ground water level, spring or seepage water enters, and the stream becomes permanent. An aquatic flora and fauna develops and water flows the year round. Youthful streams typically have a relatively steep gradient, rocky beds, with rapids, falls, and small pools.

3 Mature streams. Mature streams have wide valleys, a developed flood plain, are deeper, more turbid, and usually have warmer water, sand, mud, silt, or clay bottom materials which shift with increase in flow. In their more favorable reaches, streams in this condition are at a peak of biological productivity. Gradients are moderate, riffles or rapids are often separated by long pools.

4 In old age, streams have approached geologic base level, usually the ocean. During flood stage they scour their beds and deposit materials on the flood plain which may be very broad and flat. During normal flow the channel is refilled and many shifting bars are developed.

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(Under the influence of man this pattern may be broken up, or temporarily interrupted. Thus an essentially "youthful" stream might take on some of the characteristics of a "mature" stream following soil erosion, organic enrichment, and increased surface runoff. Correction of these conditions might likewise be followed by at least a partial reversion to the "original" condition).

C Lakes and Reservoirs

Geological factors which significantly affect the nature of either a stream or lake include the following:

1 The geographical location of the drainage basin or watershed.

2 The size and shape of the drainage basin.

3 The general topography, i.e., mountainous or plains.

4 The character of the bedrocks and soils.

5 The character, amount, annual distribution, and rate of precipitation.

6 The natural vegetative cover of the land is, of course, responsive to and responsible for many of the above factors and is also severely subject to the whims of civilization. This is one of the major factors determining run-off versus soil absorption, etc.

D Lakes have a developmental history which somewhat parallels that of streams. This process is often referred to as natural eutrophication.

1 The methods of formation vary greatly, but all influence the character and subsequent history of the lake.

In glaciated areas, for example, a huge block of ice may have been covered with till. The glacier retreated, the ice melted, and the resulting hole became a lake. Or, the glacier may actually scoop out a hole. Landslides may dam valleys, extinct volcanoes may collapse, etc., etc.

2 Maturing or natural eutrophication of lakes.

a If not already present shoal areas are developed through erosion and deposition of the shore material by wave action and undertow.

b Currents produce bars across bays and thus cut off irregular areas.

c Silt brought in by tributary streams settles out in the quiet lake water

d Algae grow attached to surfaces, and floating free as plankton. Dead organic matter begins to accumulate on the bottom.

e Rooted aquatic plants grow on shoals and bars, and in doing so cut off bays and contribute to the filling of the lake.

f Dissolved carbonates and other materials are precipitated in the deeper portions of the lake in part through the action of plants.

g When filling is well advanced, mats of sphagnum moss may extend outward from the shore. These mats are followed by sedges and grasses which finally convert the lake into a marsh.

3 Extinction of lakes. After lakes reach maturity, their progress toward filling up is accelerated. They become extinct through:

a The downcutting of the outlet.

b Filling with detritus eroded from the shores or brought in by tributary streams.

c Filling by the accumulation of the remains of vegetable materials growing in the lake itself. (Often two or three processes may act concurrently)

III PRODUCTIVITY IN FRESH WATERS

A Fresh waters in general and under natural conditions by definition have a lesser supply of dissolved substances than marine waters, and thus a lesser basic potential for the growth of aquatic organisms. By the same token, they may be said to be more sensitive to the addition of extraneous materials (pollutants, nutrients, etc.) The following notes are directed toward natural geological and other environmental factors as they affect the productivity of fresh waters.

B Factors Affecting Stream Productivity (See Table 1)

TABLE 1

EFFECT OF SUBSTRATE ON STREAM PRODUCTIVITY*

(The productivity of sand bottoms is taken as 1)

Bottom Material	Relative Productivity
Sand	1
Marl	6
Fine Gravel	9
Gravel and silt	14
Coarse gravel	32
Moss on fine gravel	89
Fissidens (moss) on coarse gravel	111
Ranunculus (water buttercup)	194
Watercress	301
Anacharis (waterweed)	452

*Selected from Tarzwell 1937

To be productive of aquatic life, a stream must provide adequate nutrients, light, a suitable temperature, and time for growth to take place.

1 Youthful streams, especially on rock or sand substrates are low in essential nutrients. Temperatures in mountainous regions are usually low, and due to the steep gradient, time for growth is short. Although ample light is available, growth of true plankton is thus greatly limited.

2 As the stream flows toward a more "mature" condition, nutrients tend to accumulate, and gradient diminishes and so time of flow increases, temperature tends to increase, and plankton flourish.

Should a heavy load of inert silt develop on the other hand, the turbidity would reduce the light penetration and consequently the general plankton production would diminish.

3 As the stream approaches base level (old age) and the time available for plankton growth increases, the balance between turbidity, nutrient levels, and temperature and other seasonal conditions, determines the overall productivity.

C Factors Affecting the Productivity of lakes (See Table 2)

1 The size, shape, and depth of the lake basin. Shallow water is more productive than deeper water since more light will reach the bottom to stimulate rooted plant growth. As a corollary, lakes with more shoreline, having more shallow water, are in general more productive. Broad shallow lakes and reservoirs have the greatest production potential (and hence should be avoided for water supplies).

TABLE 2

EFFECT OF SUBSTRATE ON LAKE PRODUCTIVITY *

(The productivity of sand bottoms is taken as 1)

Bottom Material	Relative Productivity
Sand	1
Pebbles	4
Clay	8
Flat rubble	9
Block rubble	11
Shelving rock	77

* Selected from Tarzwell 1937

2 Hard waters are generally more productive than soft waters as there are more plant nutrient minerals available. This is often greatly influenced by the character of the soil and rocks in the watershed and the quality and quantity of ground water entering the lake. In general, pH ranges of 6.8 to 8.2 appear to be most productive.

3 Turbidity reduces productivity as light penetration is reduced.

4 The presence or absence of thermal stratification with its semi-annual turnovers affects productivity by distributing nutrients throughout the water mass.

5 Climate, temperature, prevalence of ice and snow, are also of course important.

D Factors Affecting the Productivity of Reservoirs

1 The productivity of reservoirs is governed by much the same principles as that of lakes, with the difference that the water level is much more under the control of man. Fluctuations in water level can be used to deliberately increase or decrease productivity. This can be demonstrated by a comparison of the TVA reservoirs which practice a summer drawdown with some of those in the west where a winter drawdown is the rule.

2 The level at which water is removed from a reservoir is important to the productivity of the stream below. The hypolimnion may be anaerobic while the epilimnion is aerobic, for example, or the epilimnion is poor in nutrients while the hypolimnion is relatively rich.

3 Reservoir discharges also profoundly affect the DO, temperature, and turbidity in the stream below a dam. Too much fluctuation in flow may permit sections of the stream to dry, or provide inadequate dilution for toxic waste.

IV CULTURAL EUTROPHICATION

A The general processes of natural eutrophication, or natural enrichment and productivity have been briefly outlined above.

B When the activities of man speed up these enrichment processes by introducing unnatural quantities of nutrients (sewage, etc.) the result is often called cultural eutrophication. This term is often extended beyond its original usage to include the enrichment (pollution) of streams, estuaries, and even oceans, as well as lakes.

V CLASSIFICATION OF LAKES AND RESERVOIRS

A The productivity of lakes and impoundments is such a conspicuous feature that it is often used as a convenient means of classification.

1 Oligotrophic lakes are the younger, less productive lakes, which are deep, have clear water, and usually support Salmonoid fishes in their deeper waters.

2 Eutrophic lakes are more mature, more turbid, and richer. They are usually shallower. They are richer in dissolved solids; N, P, and Ca are abundant. Plankton is abundant and there is often a rich bottom fauna.

3 Dystrophic lakes, such as bog lakes, are low in Ph, water yellow to brown, dissolved solids, N, P, and Ca scanty but humic materials abundant, bottom fauna and plankton poor, and fish species are limited.

B Reservoirs may also be classified as storage, and run of the river.

1 Storage reservoirs have a large volume in relation to their inflow.

2 Run of the river reservoirs have a large flow-through in relation to their storage value.

C According to location, lakes and reservoirs may be classified as polar, temperate, or tropical. Differences in climatic and geographic conditions result in differences in their biology.

VI SUMMARY

A A body of water such as a lake, stream, or estuary represents an intricately balanced system in a state of dynamic equilibrium. Modification imposed at one point in the system automatically results in compensatory adjustments at associated points.

B The more thorough our knowledge of the entire system, the better we can judge where to impose control measures to achieve a desired result.

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Descriptors:

Aquatic Environment, Estuarine Environment, Lentic Environment, Lotic Environment, Currents, Marshes, Limnology, Water Properties

THE AQUATIC ENVIRONMENT

Part 4. The Marine Environment and its Role in the Total Aquatic Environment

I INTRODUCTION

A The marine environment is arbitrarily defined as the water mass extending beyond the continental land masses, including the plants and animals harbored therein. This water mass is large and deep, covering about 70 percent of the earth's surface and being as deep as 7 miles. The salt content averages about 35 parts per thousand. Life extends to all depths.

B The general nature of the water cycle on earth is well known. Because the largest portion of the surface area of the earth is covered with water, roughly 70 percent of the earth's rainfall is on the seas. (Figure 1)

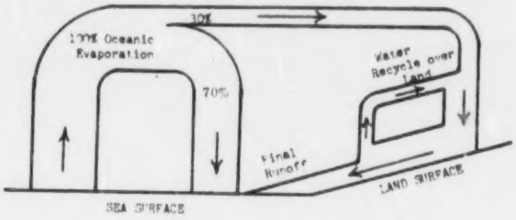

Figure 1. THE WATER CYCLE

Since roughly one third of the rain which falls on the land is again recycled through the atmosphere (see Figure 1 again), the total amount of water washing over the earth's surface is significantly greater than one third of the total world rainfall. It is thus not surprising to note that the rivers which finally empty into the sea carry a disproportionate burden of dissolved and suspended solids picked up from the land. The chemical composition of this burden depends on the composition of the rocks and soils through which the river flows, the proximity of an ocean, the direction of prevailing winds, and other factors. This is the substance of geological erosion. (Table 1)

TABLE 1

PERCENTAGE COMPOSITION OF THE MAJOR IONS OF TWO STREAMS AND SEA WATER

(Data from Clark, F.W., 1924, "The Composition of River and Lake Waters of the United States", U.S. Geol. Surv., Prof. Paper No. 135; Harvey, H.W., 1957, "The Chemistry and Fertility of Sea Waters", Cambridge University Press, Cambridge)

Ion	Delaware River at Lambertville, N.J.	Rio Grande at Laredo, Texas	Sea Water
Na	6.70	14.78	30.4
K	1.46	.85	1.1
Ca	17.49	13.73	1.16
Mg	4.81	3.03	3.7
Cl	4.23	21.65	55.2
SO_4	17.49	30.10	7.7
CO_3	32.95	11.55	+HCO_3 0.35

C For this presentation, the marine environment will be (1) described using an ecological approach, (2) characterized ecologically by comparing it with freshwater and estuarine environments, and (3) considered as a functional ecological system (ecosystem).

II FRESHWATER, ESTUARINE, AND MARINE ENVIRONMENTS

Distinct differences are found in physical, chemical, and biotic factors in going from a freshwater to an oceanic environment. In general, environmental factors are more constant in freshwater (rivers) and oceanic environments than in the highly variable and harsh environments of estuarine and coastal waters. (Figure 2)

A Physical and Chemical Factors

Rivers, estuaries, and oceans are compared in Figure 2 with reference to the relative instability (or variation) of several important parameters. In the oceans, it will be noted, very little change occurs in any parameter. In rivers, while "salinity" (usually referred to as "dissolved solids") and temperature (accepting normal seasonal variations) change little, the other four parameters vary considerably. In estuaries, they all change.

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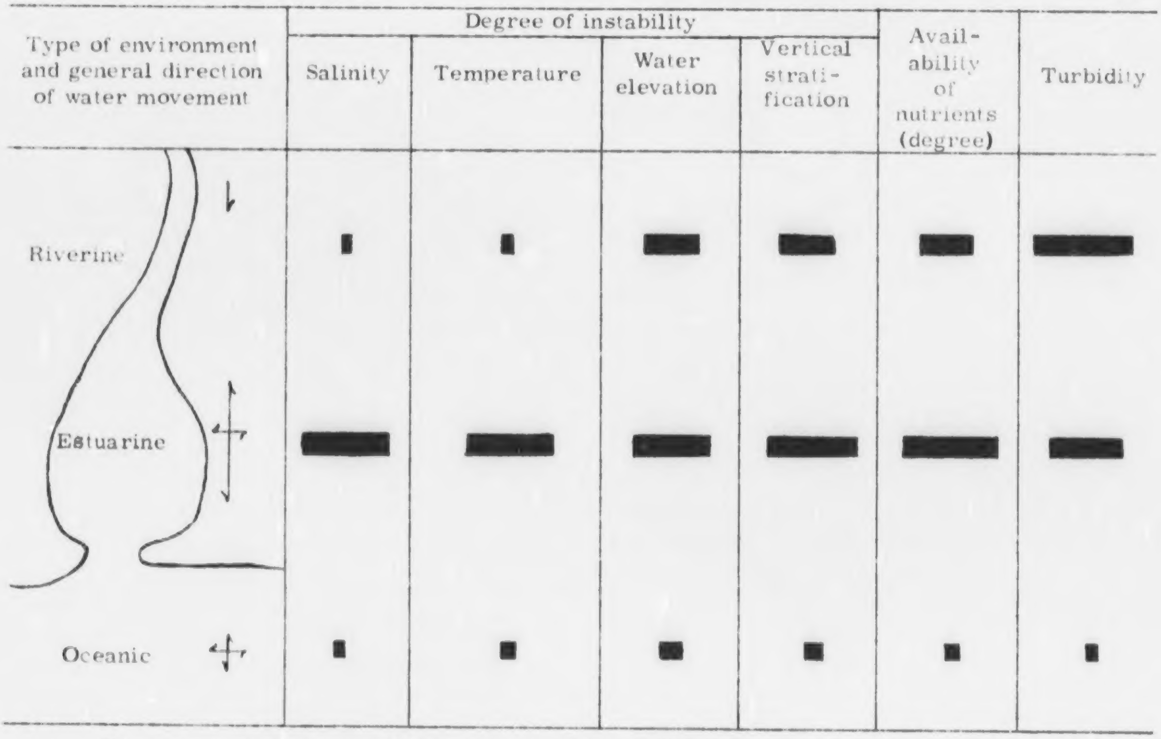

Figure 2. RELATIVE VALUES OF VARIOUS PHYSICAL AND CHEMICAL FACTORS FOR RIVER, ESTUARINE, AND OCEANIC ENVIRONMENTS

B Biotic Factors

1 A complex of physical and chemical factors determine the biotic composition of an environment. In general, the number of species in a rigorous, highly variable environment tends to be less than the number in a more stable environment (Hedgpeth, 1966).

2 The dominant animal species (in terms of total biomass) which occur in estuaries are often transient, spending only a part of their lives in the estuaries. This results in better utilization of a rich environment.

C Zones of the Sea

The nearshore environment is often classified in relation to tide level and water depth. The nearshore and offshore oceanic regions together, are often classified with reference to light penetration and water depth. (Figure 3)

1 Neritic - Relatively shallow-water zone which extends from the high-tide mark to the edge of the continental shelf.

MARINE ECOLOGY

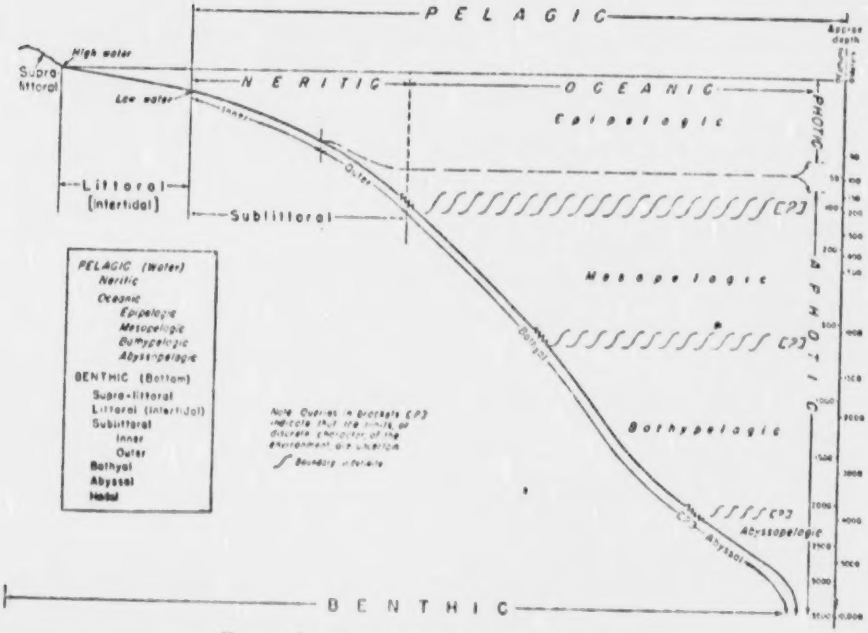
PELAGIC
Approx depth
Supra-littoral
High water
Low water
NERITIC
OCEANIC
Epipelagic
PHOTIC
Inner
Outer
Littoral [Intertidal]
Sublittoral
[?]
Mesopelagic
Bathyal
[?]
APHOTIC
Bathypelagic
[?]
Abyssopelagic
[?]
Abyssal
PELAGIC (Water)
Neritic
Oceanic
Epipelagic
Mesopelagic
Bathypelagic
Abyssopelagic
BENTHIC (Bottom)
Supra-littoral
Littoral (Intertidal)
Sublittoral
Inner
Outer
Bathyal
Abyssal
Hadal
Note: Queries in brackets [?] indicate that the limits or discrete character of the environment are uncertain
Boundary indefinite
1000
1500
2000
3000
4000
BENTHIC

FIGURE 3—*Classification of marine environments*

a Stability of physical factors is intermediate between estuarine and oceanic environments.

b Phytoplankters are the dominant producers but in some locations attached algae are also important as producers.

c The animal consumers are zooplankton, nekton, and benthic forms.

2 Oceanic - The region of the ocean beyond the continental shelf. Divided into three parts, all relatively poorly populated compared to the neritic zone.

a Euphotic zone - Waters into which sunlight penetrates (often to the bottom in the neritic zone). The zone of primary productivity often extends to 600 feet below the surface.

1) Physical factors fluctuate less than in the neritic zone.

2) Producers are the phytoplankton and consumers are the zooplankton and nekton.

b Bathyal zone - From the bottom of the euphotic zone to about 2000 meters.

1) Physical factors relatively constant but light is absent.

2) Producers are absent and consumers are scarce.

c Abyssal zone - All the sea below the bathyal zone.

1) Physical factors more constant than in bathyal zone.

2) Producers absent and consumers even less abundant than in the bathyal zone.

III SEA WATER AND THE BODY FLUIDS

A Sea water is a remarkably suitable environment for living cells, as it contains all of the chemical elements essential to the growth and maintenance of plants and animals. The ratio and often the concentration of the major salts of sea water are strikingly similar in the cytoplasm and body fluids of marine organisms. This similarity is also evident, although modified somewhat in the body fluids of fresh water and terrestrial animals. For example, sterile sea water may be used in emergencies as a substitute for blood plasma in man.

B Since marine organisms have an internal salt content similar to that of their surrounding medium (isotonic condition) osmoregulation poses no problem. On the other hand, fresh water organisms are hypertonic (osmotic pressure of body fluids is higher than that of the surrounding water). Hence, fresh water animals must constantly expend more energy to keep water out (i.e., high osmotic pressure fluids contain more salts, the action being then to dilute this concentration with more water).

1 Generally, marine invertebrates are narrowly poikilosmotic, i.e., the salt concentration of the body fluids changes with that of the external medium. This has special significance in estuarine situations where salt concentrations of the water often vary considerably in short periods of time.

2 Marine bony fish (teleosts) have lower salt content internally than the external environment (hypotonic). In order to prevent dehydration, water is ingested and salts are excreted through special cells in the gills.

IV FACTORS AFFECTING THE DISTRIBUTION OF MARINE AND ESTUARINE ORGANISMS

A Salinity. Salinity is the single most constant and controlling factor in the marine environment, probably followed by temperature. It ranges around 35,000 mg. per liter, or "35 parts per thousand" (symbol: 35‰) in the language of the oceanographer. While variations in the open ocean are relatively small, salinity decreases rapidly as one approaches shore and proceeds through the estuary and up into fresh water with a salinity of "0 ‰ (see Figure 2)

B Salinity and temperature as limiting factors in ecological distribution.

1 Organisms differ in the salinities and temperatures in which they prefer to live, and in the variabilities of these parameters which they can tolerate. These preferences and tolerances often change with successive life history stages, and in turn often dictate where the organisms live: their "distribution."

2 These requirements or preferences often lead to extensive migrations of various species for breeding, feeding, and growing stages. One very important result of this is that an estuarine environment is an absolute necessity for over half of all coastal commercial and sport related species of fishes and invertebrates, for either all or certain portions of their life histories. (Part V, figure 8)

3 The Greek word roots "eury" (meaning wide) and "steno" (meaning narrow) are customarily combined with such words as "haline" for salt, and "thermal" for temperature, to give us "euryhaline" as an adjective to characterize an organism able to tolerate a wide range of salinity, for example; or "stenothermal" meaning one which cannot stand much change in temperature. "Meso-" is a prefix indicating an intermediate capacity.

C Marine, estuarine, and fresh water organisms. (See Figure 4)

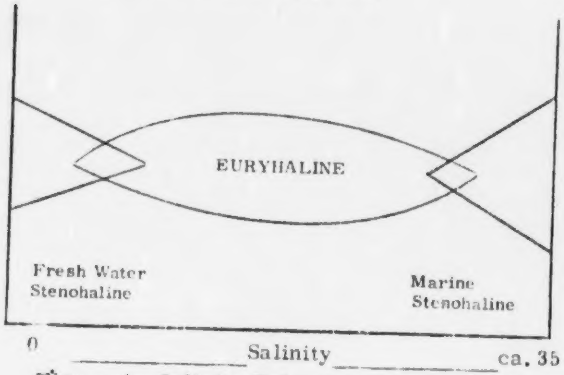

Figure 4. Salinity Tolerance of Organisms

1 Offshore marine organisms are, in general, both stenohaline and stenothermal unless, as noted above, they have certain life history requirements for estuarine conditions.

2 Fresh water organisms are also stenohaline, and (except for seasonal adaptation) meso- or stenothermal. (Figure 2)

3 Indigenous or native estuarine species that normally spend their entire lives in the estuary are relatively few in number. (See Figure 5). They are generally meso- or euryhaline and meso- or eurythermal.

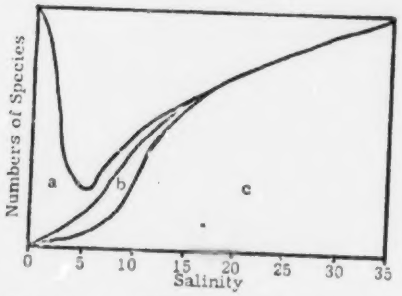

Figure 5. DISTRIBUTION OF ORGANISMS IN AN ESTUARY

a Euryhaline, freshwater
b Indigenous, estuarine, (mesohaline)
c Euryhaline, marine

4 Some well known and interesting examples of migratory species which change their environmental preferences with the life history stage include the shrimp (mentioned above), striped bass, many herrings and relatives, the salmons, and many others. None are more dramatic than the salmon hordes which lay their eggs in freshwater streams, migrate far out to sea to feed and grow, then return to the stream where they hatched to lay their own eggs before dying.

5 Among euryhaline animals landlocked (trapped), populations living in lowered salinities often have a smaller maximum size than individuals of the same species living in more saline waters. For example, the lamprey (Petromyzon marinus) attains a length of 30 - 36" in the sea, while in the Great Lakes the length is 18 - 24".

Usually the larvae of aquatic organisms are more sensitive to changes in salinity than are the adults. This characteristic both limits and dictates the distribution and size of populations.

D The effects of tides on organisms.

1 Tidal fluctuations probably subject the benthic or intertidal populations to the most extreme and rapid variations of environmental stress encountered in any aquatic habitat. Highly specialized communities have developed in this zone, some adapted to the rocky surf zones of the open coast, others to the muddy inlets of protected estuaries. Tidal reaches of fresh water rivers, sandy beaches, coral reefs and mangrove swamps in the tropics; all have their own floras and faunas. All must emerge and flourish when whatever water there is rises and covers or tears at them, all must collapse or retract to endure drying, blazing tropical sun, or freezing arctic ice during the low tide interval. Such a community is depicted in Figure 6.

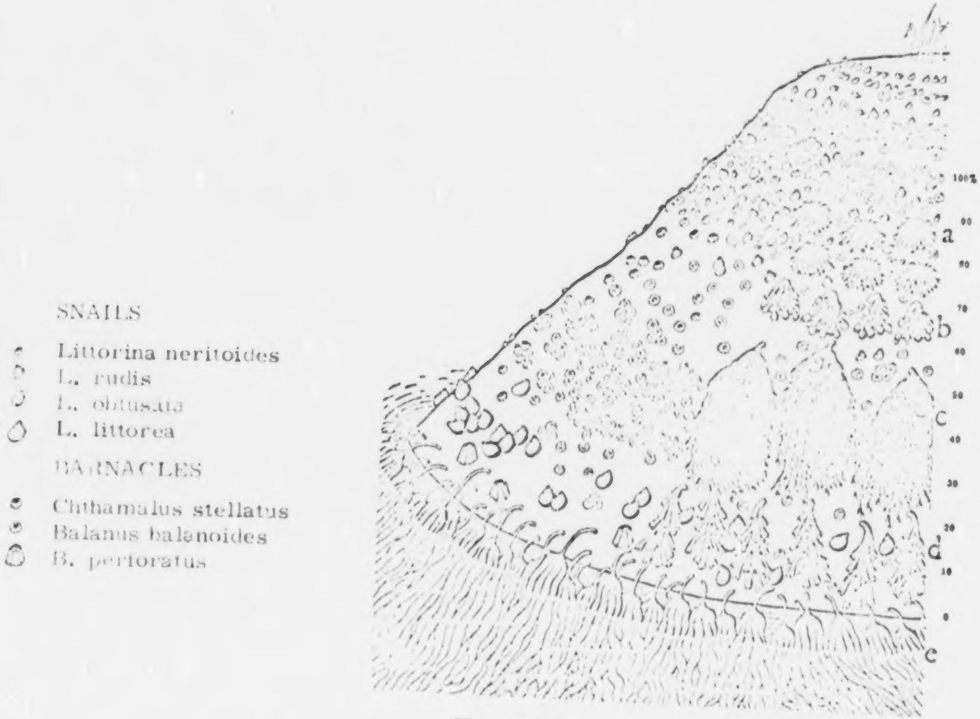
SNAILS
Littorina neritoides
L. rudis
L. obtusata
L. littorea
BARNACLES
Chthamalus stellatus
Balanus balanoides
B. perforatus
100%
90
a
80
70
b
60
50
c
40
30
20
d
10
0
e

Figure 6

Zonation of plants, snails, and barnacles on a rocky shore. While this diagram is based on the situation on the southwest coast of England, the general idea of zonation may be applied to any temperate rocky ocean shore, though the species will differ. The gray zone consists largely of lichens. At the left is the zonation of rocks with exposure too extreme to support algae; at the right, on a less exposed situation, the animals are mostly obscured by the algae. Figures at the right hand margin refer to the percent of time that the zone is exposed to the air, i.e., the time that the tide is out. Three major zones can be recognized: the Littorina zone (above the gray zone); the Balanoid zone (between the gray zone and the laminarias); and the Laminaria zone. a. Pelvetia canaliculata; b. Fucus spiralis; c. Ascophyllum nodosum; d. Fucus serratus; e. Laminaria digitata. (Based on Stephenson)

V FACTORS AFFECTING THE PRODUCTIVITY OF THE MARINE ENVIRONMENT

A The sea is in continuous circulation. Without circulation, nutrients of the ocean would eventually become a part of the bottom and biological production would cease. Generally, in all oceans there exists a warm surface layer which overlies the colder water and forms a two-layer system of persistent stability. Nutrient concentration is usually greatest in the lower zone. Wherever a mixing or disturbance of these two layers occurs biological production is greatest.

B The estuaries are also a mixing zone of enormous importance. Here the fertility washed off the land is mingled with the nutrient capacity of seawater, and many of the would's most productive waters result.

C When man adds his cultural contributions of sewage, fertilizer, silt or toxic waste, it is no wonder that the dynamic equilibrium of the ages is rudely upset, and the environmentalist cries, "See what man hath wrought"!

ACKNOWLEDGEMENT:

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Descriptors:
Aquatic Environment, Estuarine Environment, Lentic Environment, Lotic Environment, Currents, Marshes, Limnology, Water Properties

THE AQUATIC ENVIRONMENT

Part 5: Wetlands

I INTRODUCTION

A Broadly defined, wetlands are areas which are "to wet to plough but too thick to flow." The soil tends to be saturated with water, salt or fresh, and numerous channels or ponds of shallow or open water are common. Due to ecological features too numerous and variable to list here, they comprise in general a rigorous (highly stressed) habitat, occupied by a small relatively specialized indigenous (native) flora and fauna.

B They are prodigiously productive however, and many constitute an absolutely essential habitat for some portion of the life history of animal forms generally recognized as residents of other habitats (Figure 8). This is particularly true of tidal marshes as mentioned below.

C Wetlands in toto comprise a remarkably large proportion of the earth's surface, and the total organic carbon bound in their mass constitutes an enormous sink of energy.

D Since our main concern here is with the "aquatic" environment, primary emphasis will be directed toward a description of wetlands as the transitional zone between the waters and the land, and how their desecration by human culture spreads degradation in both directions.

II TIDAL MARSHES AND THE ESTUARY

A "There is no other case in nature, save in the coral reefs, where the adjustment of organic relations to physical condition is seen in such a beautiful way as the balance between the growing marshes and the tidal streams by which they are at once nourished and worn away." (Shaler, 1886)

B Estuarine pollution studies are usually devoted to the dynamics of the circulating water, its chemical, physical, and biological parameters, bottom deposits, etc.

C It is easy to overlook the intimate relationships which exist between the bordering marshland, the moving waters, the tidal flats, subtidal deposition, and seston whether of local, oceanic, or riverine origin.

D The tidal marsh (some inland areas also have salt marshes) is generally considered to be the marginal areas of estuaries and coasts in the intertidal zone, which are dominated by emergent vegetation. They generally extend inland to the farthest point reached by the spring tides, where they merge into freshwater swamps and marshes (Figure 1). They may range in width from nonexistent on rocky coasts to many kilometers.

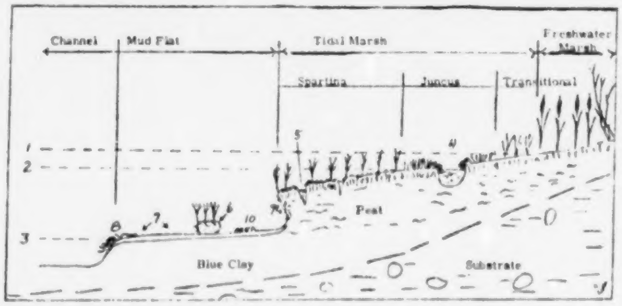

Figure 1. Zonation in a positive New England estuary. 1. Spring tide level, 2. Mean high tide, 3. Mean low tide, 4. Bog hole, 5. Ice cleavage pool, 6. Chunk of Spartina turf deposited by ice, 7. Organic ooze with associated community, 8. eelgrass (Zostera), 9. Ribbed mussels (modiolus)-clam (mya) - mud snail (Nassa) community. 10. Sea lettuce (Ulva)

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III MARSH ORIGINS AND STRUCTURES

A In general, marsh substrates are high in organic content, relatively low in minerals and trace elements. The upper layers bound together with living roots called turf, underlaid by more compacted peat type material.

1 Rising or eroding coastlines may expose peat from ancient marsh growth to wave action which cuts into the soft peat rapidly (Figure 2). Such banks are likely to be cliff-like, and are often undercut. Chunks of peat are often found lying about on harder substrate below high tide line. If face of cliff is well above high water, overlying vegetation is likely to be typically terrestrial of the area. Marsh type vegetation is probably absent.

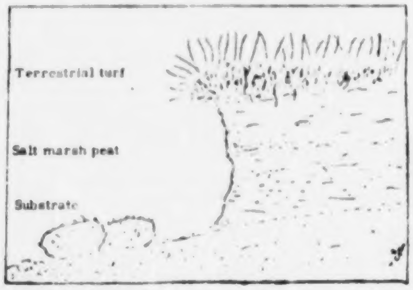

Figure 2 Diagrammatic section of eroding peat cliff

2 Low lying deltaic, or sinking coastlines, or those with low energy wave action are likely to have active marsh formation in progress. Sand dunes are also common in such areas (Figure 3). General coastal configuration is a factor.

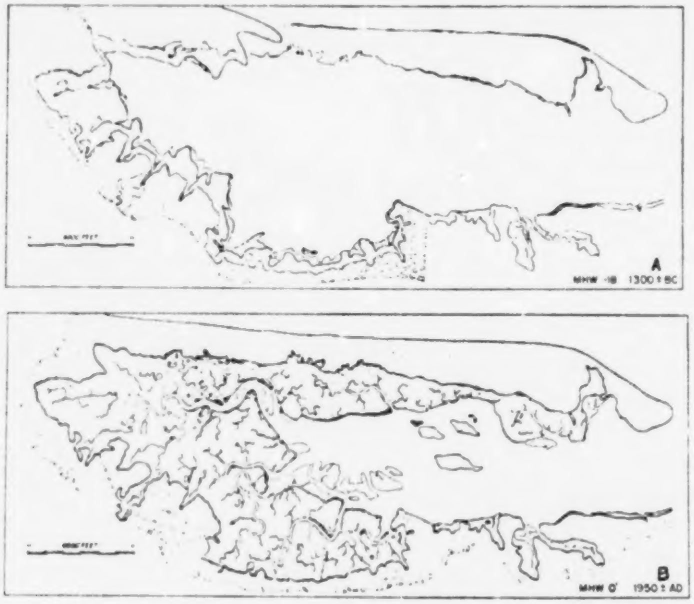

Figure 3

Development of a Massachusetts Marsh since 1300 BC, involving an 18 foot rise in water level. Shaded area indicates sand dunes. Note meandering marsh tidal drainage. A: 1300 BC, B: 1950 AD.

a Rugged or precipitous coasts or slowly rising coasts, typically exhibit narrow shelves, sea cliffs, fjords, massive beaches, and relatively less marsh area (Figure 4). An Alaskan fjord subject to recent catastrophic subsidence and rapid deposition of glacial flour shows evidence of the recent encroachment of saline waters in the presence of recently buried trees and other terrestrial vegetation, exposure of layers of salt marsh peat along the edges of channels, and a poorly compacted young marsh turf developing at the new high water level (Figure 5).

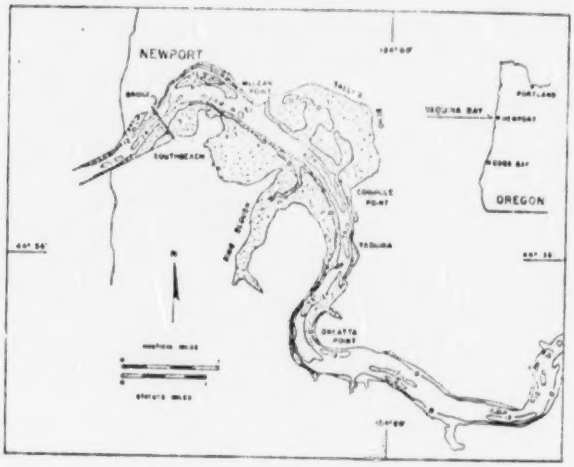

Figure 4 A River Mouth on a Slowly Rising Coast. Note absence of deltaic development and relatively little marshland, although mud flats stippled are extensive.

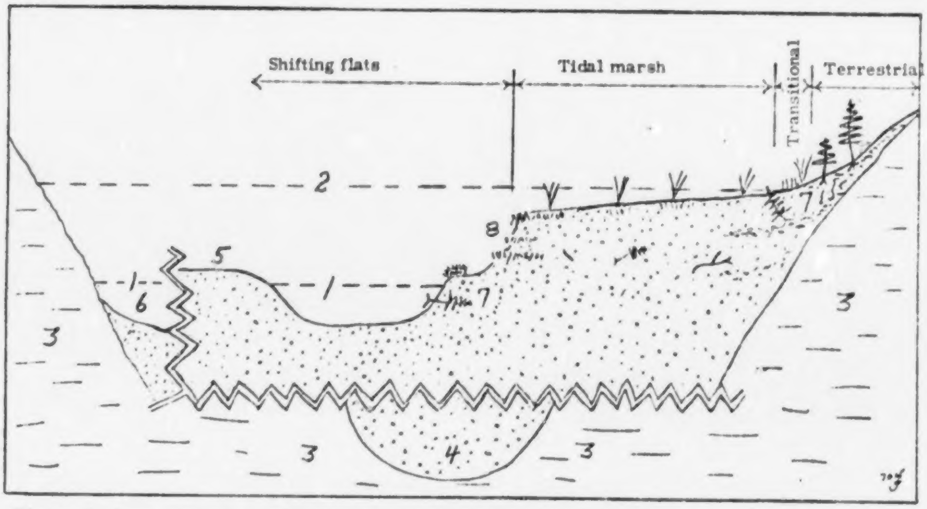

Figure 5 Some general relationships in a northern fjord with a rising water level. 1. mean low water, 2. maximum high tide, 3. Bedrock, 4. Glacial flour to depths in excess of 400 meters, 5. Shifting flats and channels, 6. Channel against bedrock, 7. Buried terrestrial vegetation, 8. Outcroppings of salt marsh peat.

b Low lying coastal plains tend to be fringed by barrier islands, broad estuaries and deltas, and broad associated marshlands (Figure 3).

Deep tidal channels fan out through innumerable branching and often interconnecting rivulets. The intervening grassy plains are essentially at mean high tide level.

c Tropical and subtropical regions such as Florida, the Gulf Coast, and Central America, are frequented by mangrove swamps. This unique type of growth is able to establish itself in shallow water and move out into progressively deeper areas (Figure 6). The strong deeply embedded roots enable the mangrove to resist considerable wave action at times, and the tangle of roots quickly accumulates a deep layer of organic sediment. Mangroves in the south may be considered to be roughly the equivalent of the Spartina marsh grass in the north as a land builder. When fully developed, a mangrove swamp is an impenetrable thicket of roots over the tidal flat affording shelter to an assortment of semi-aquatic organisms such as various molluscs and crustaceans, and providing access from the nearby land to predaceous birds, reptiles, and mammals. Mangroves are not restricted to estuaries, but may develop out into shallow oceanic lagoons, or upstream into relatively fresh waters.

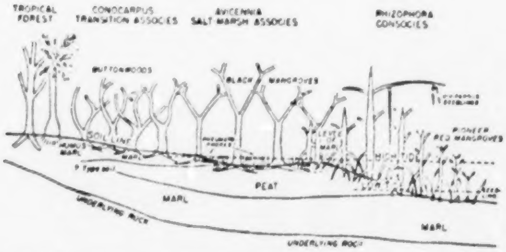

Figure 6 Diagrammatic transect of a mangrove swamp showing transition from marine to terrestrial habitat.

IV PRODUCTIVITY OF WETLANDS

A Measuring the productivity of grasslands is not easy, because today grass is seldom used directly as such by man. It is thus usually expressed as production of meat, milk, or in the case of salt marshes, the total crop of animals that obtain food per unit of area. The primary producer in a tidal marsh is the marsh grass, but very little of it is used by man as grass. (Table 1)

The nutritional analysis of several marsh grasses as compared to dry land hay is shown in Table 2.

TABLE 1. General Orders of Magnitude of Gross Primary Productivity in Terms of Dry Weight of Organic Matter Fixed Annually

Ecosystem	gms/M^2/year (grams/square meters/year)	lbs/acre/year
Land deserts, deep oceans	Tens	Hundreds
Grasslands, forests, eutrophic lakes, ordinary agriculture	Hundreds	Thousands
Estuaries, deltas, coral reefs, intensive agriculture (sugar cane, rice)	Thousands	Ten-thousands

TABLE 2. Analyses of Some Tidal Marsh Grasses

T/A	Percentage Composition					
Dry Wt	Protein	Fat	Fiber	Water	Ash	N-free Extract
Distichlis spicata (pure stand, dry)						
2.8	5.3	1.7	32.4	8.2	6.7	45.5
Short *Spartina alterniflora* and *Salicornia europaea* (in standing water)						
1.2	7.7	2.5	31.1	8.8	12.0	37.7
Spartina alterniflora (tall, pure stand in standing water)						
3.5	7.6	2.0	29.0	8.3	15.5	37.3
Spartina patens (pure stand, dry)						
3.2	6.0	2.2	30.0	8.1	9.0	44.5
Spartina alterniflora and *Spartina patens* (mixed stand, wet)						
3.4	6.8	1.9	29.8	8.1	10.4	42.8
Spartina alterniflora (short, wet)						
2.2	8.8	2.4	30.4	8.7	13.3	36.3
Comparable Analyses for Hay						
1st cut	6.0	2.0	36.2	6.7	4.2	44.9
2nd cut	11.0	3.7	28.5	10.4	5.9	30.5

Analyses performed by Roland W. Gilbert, Department of Agricultural Chemistry, U.R.I.

B The actual utilization of marsh grass is accomplished primarily by its decomposition and ingestion by micro organisms. (Figure 7) A small quantity of seeds and solids is consumed directly by birds.

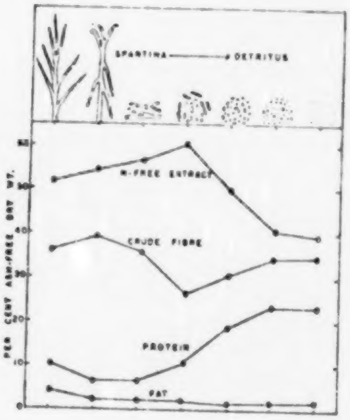

Figure 7 The nutritive composition of successive stages of decomposition of Spartina marsh grass, showing increase in protein and decrease in carbohydrate with increasing age and decreasing size of detritus particles.

1 The quantity of micro invertebrates which thrive on this wealth of decaying marsh has not been estimated, nor has the actual production of small indigenous fishes and invertebrates such as the top minnows (Fundulus), or the mud snails (Nassa), and others.

2 Many forms of oceanic life migrate into the estuaries, especially the marsh areas, for important portions of their life histories as is mentioned elsewhere (Figure 8). It has been estimated that in excess of 60% of the marine commercial and sport fisheries are estuarine or marsh dependent in some way.

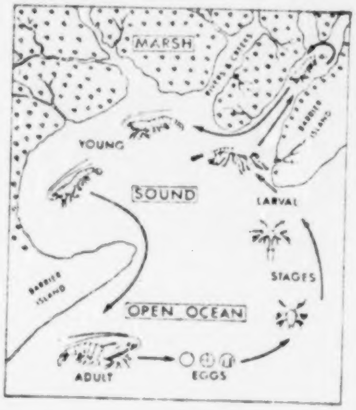

Figure 8 Diagram of the life cycle of white shrimp (after Anderson and Lunz 1965).

3 An effort to make an indirect estimate of productivity in a Rhode Island marsh was made on a single August day by recording the numbers and kinds of birds that fed on a relatively small area (Figure 9). Between 700 and 1000 wild birds of 12 species, ranging from 100 least sandpipers to uncountable numbers of seagulls were counted. One food requirement estimate for three-pound poultry in the confined inactivity of a poultry yard is approximately one ounce per pound of bird per day.

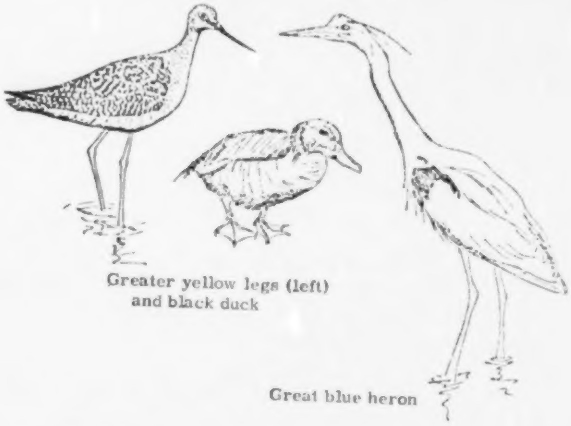

Figure 9 Some Common Marsh Birds

One-hundred black bellied plovers at approximately ten ounces each would weigh on the order of sixty pounds. At the same rate of food consumption, this would indicate nearly four pounds of food required for this species alone. The much greater activity of the wild birds would obviously greatly increase their food requirements, as would their relatively smaller size.

Considering the range of foods consumed, the sizes of the birds, and the fact that at certain seasons, thousands of migrating ducks and others pause to feed here, the enormous productivity of such a marsh can be better understood.

V INLAND BOGS AND MARSHES

A Much of what has been said of tidal marshes also applies to inland wetlands. As was mentioned earlier, not all inland swamps are salt-free, any more than all marshes affected by tidal rythms are saline.

B The specificity of specialized floras to particular types of wetlands is perhaps more spectacular in freshwater wetlands than in the marine, where *Juncus*, *Spartina*, and Mangroves tend to dominate.

1 *Sphagnum*, or peat moss, is probably one of the most widespead and abundant wetland plants on earth. Deevey (1958) quotes an estimate that there is probably upwards of 223 billions (dry weight) of tons of peat in the world today, derived during recent geologic time from *Sphagnum* bogs. Particularly in the northern regions, peat moss tends to overgrow ponds and shallow depressions, eventually forming the vast tundra plains and moores of the north.

2 Long lists of other bog and marsh plants might be cited, each with its own special requirements, topographical, and geographic distribution, etc. Included would be the familiar cattails, spike rushes, cotton grasses, sedges, trefoils, alders, and many, many others.

C Types of inland wetlands.

1 As noted above (Cf: Figure 1) tidal marshes often merge into freshwater marshes and bayous. Deltaic tidal swamps and marshes are often saline in the seaward portion, and fresh in the landward areas.

2 River bottom wetlands differ from those formed from lakes, since wide flood plains subject to periodic inundation are the final stages of the erosion of river valleys, whereas lakes in general tend to be eliminated by the geologic processes of natural eutrophication often involving *Sphagnum* and peat formation. Riverbottom marshes in the southern United States, with favorable climates, have luxurient growths such as the canebrake of the lower Mississippi, or a characteristic timber growth such as cypress.

3 Although bird life is the most conspicuous animal element in the fauna (Cf: Figure 9), many mammals, such as muskrats, beavers, otters, and others are also marsh-oriented. (Figure 12)

Figure 12

VI POLLUTION

A No single statement can summarize the effects of pollution on marshlands as distinct from effects noted elsewhere on other habitats.

B Reduction of Primary Productivity

The primary producers in most wetlands are the grasses and peat mosses. Production may be reduced or eliminated by:

1 Changes in the water level brought about by flooding or drainage.

a Marshland areas are sometimes diked and flooded to produce fresh-water ponds. This may be for aesthetic reasons, to suppress the growth of noxious marsh inhabitating insects such as mosquitoes or biting midges, to construct an industrial waste holding pond, a thermal or a sewage stabilization pond, a "convenient" result of highway causeway construction, or other reason. The result is the elimination of an area of marsh. A small compensating border of marsh may or may not develop.

b High tidal marshes were often ditched and drained in former days to stabilize the sod for salt hay or "thatch" harvesting which was highly sought after in colonial days. This inevitably changed the character of the marsh, but it remained as essentially marshland. Conversion to outright agricultural land has been less widespread because of the necessity of diking to exclude the periodic floods or tidal incursions, and carefully timed drainage to eliminate excess precipitation. Mechanical pumping of tidal marshes has not been economical in this country, although the success of the Dutch and others in this regard is well known.

2 Marsh grasses may also be eliminated by smothering as, for example, by deposition of dredge spoils, or the spill or discharge of sewage sludge.

3 Considerable marsh area has been eliminated by industrial construction activity such as wharf and dock construction, oil well construction and operation, and the discharge of toxic brines and other chemicals.

C Consumer production (animal life) has been drastically reduced by the deliberate distribution of pesticides. In some cases, this has been aimed at nearby agricultural lands for economic crop pest control, in other cases the marshes have been sprayed or dusted directly to control noxious insects.

1 The results have been universally disastrous for the marshes, and the benefits to the human community often questionable.

2 Pesticides designed to kill nuisance insects, are also toxic to other arthropods so that in addition to the target species, such forage staples as the various scuds (amphipods), fiddler crabs, and other macroinvertebrates have either been drastically reduced or entirely eliminated in many places. For example, one familiar with fiddler crabs can traverse miles of marsh margins, still riddled with their burrows, without seeing a single live crab.

3 DDT and related compounds have been "eaten up the food chain" (biological magnification effect) until fish eating and other predatory birds such as herons and egrets (Figure 9), have been virtually eliminated from vast areas, and the accumulation of DDT in man himself is only too well known.

D Most serious of the marsh enemies is man himself. In his quest for "lebensraum" near the water, he has all but killed the water he strives to approach. Thus up to twenty percent of the marsh--estuarine area in various parts of the country has already been utterly destroyed by cut and fill real estate developments (Figures 10, 11).

E Swimming birds such as ducks, loons, cormorants, pelicans, and many others are severely jeopardized by floating pollutants such as oil.

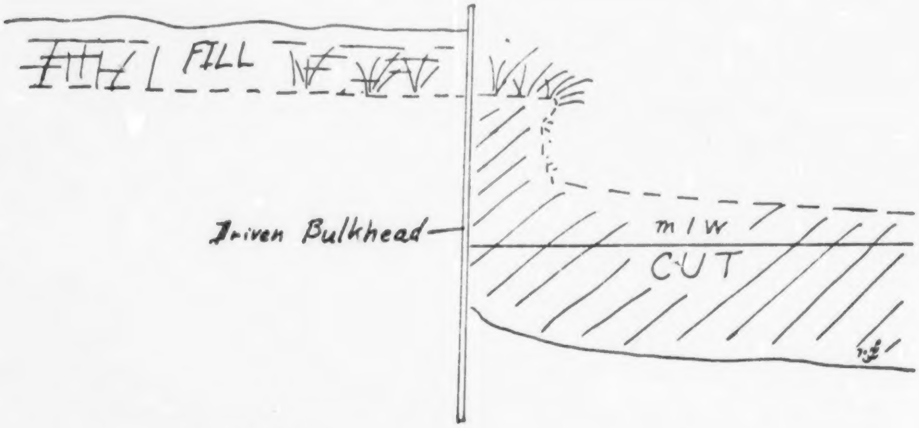
FILL
Driven Bulkhead
mlw
CUT

Figure 10. Diagrammatic representation of cut-and-fill for real estate development. mlw = mean low water

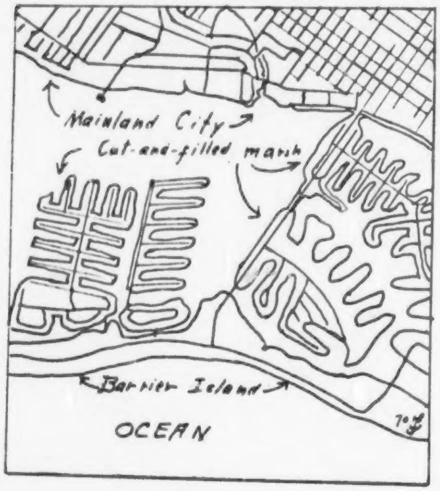
Mainland City
Cut-and-filled marsh
Barrier Island
OCEAN

Figure 11. Tracing of portion of map of a southern city showing extent of cut-and-fill real estate development.

VII SUMMARY

A Wetlands comprise the marshes, swamps, bogs, and tundra areas of the world. They are essential to the well-being of our surface waters and ground waters. They are essential to aquatic life of all types living in the open waters. They are essential as habitat for all forms of wildlife.

B The tidal marsh is the area of emergent vegetation bordering the ocean or an estuary.

C Marshes are highly productive areas, essential to the maintenance of a well rounded community of aquatic life.

D Wetlands may be destroyed by:

1 Degradation of the life forms of which it is composed in the name of nuisance control.

2 Physical destruction by cut-and-fill to create more land area.

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Descriptors: Aquatic Environment, Estuarine Environment, Lentic Environment Lotic Environment, Currents, Marshes, Limnology

THE IDENTIFICATION OF AQUATIC ORGANISMS

I INTRODUCTION

A There are few major groups of organisms that are either exclusively terrestrial or aquatic. The following remarks will therefore apply in large measure to both, but primary attention will be directed to aquatic types.

B One of the first questions usually posed about an organism is: "What is it," usually meaning "What is it's name?" The naming or classification of biological organisms is a science in itself (taxonomy). Some of the principles involved need to be understood by anyone working with organisms.

1 Names are the "key number," "code designation," or "file references" which we must have to find information about an unknown organism.

2 Why are they so long and why must they be in Latin and Greek? File references in large systems have to be long in order to designate the many divisions and subdivisions. There are over a million and a half items (or species) included in the system of biological nomenclature (very few libraries have a million books).

3 The system of biological nomenclature is regulated by international congresses.

a It is based on a system of groups and super groups, of which the foundation (which actually exists in nature) is the species. Everything else has been devised by man and is subject to change and revision as man's knowledge and understanding increase.

b The categories employed are as follows:

Similar species are grouped into genera (genus)

Similar genera are grouped into families

Similar families are grouped into orders

Similar orders are grouped into classes

Similar classes are grouped into phyla (phylum)

Similar phyla are grouped into kingdoms

4 The scientific name of an organism is its genus name plus its species name. This is analogous to our system of surnames (family names) and given names (Christian names).

a The generic (genus) name is always capitalized and the species name written with a small letter. They should also be underlined or printed in italics when used in a technical sense. For example:

Homo sapiens - modern man

Homo neanderthalis - neanderthal man

Esox niger - chain pickerel

Esox lucius - northern pike

Esox masquinongy - muskellunge

b Common names do not exist for most of the smaller and less familiar organisms. For example, if we wish to refer to members of the genus Anabaena (an alga), we must simply use the generic name, and:

Anabaena planctonica,

Anabaena constricta, and

Anabaena flos-aquae

are three distinct species which have different significances to water treatment plant operations.

5 A complete list of the various categories to which an organism belongs is known as its "classification." This may be written as follows for *Phacus pyrum* a green flagellate, for example:

Kingdom Plantae

Phylum Thallophyta

Class Algae

Order Volvocales

Family Phacotaceae

Genus *Phacus*

Species *pyrum*

a It should be emphasized that since all categories of above species are essentially human concepts, there is often divergence of opinion in regard to how certain organisms should be grouped. Changes result.

b The most appropriate or correct name for a given species is also sometimes disputed, and so species *names* too are changed. The species itself, as an entity in nature, however, is relatively timeless and so does not change to man's eye.

II KEY TO SELECTED LARGER GROUPS OF AQUATIC ANIMALS

The following key is intended to provide an introductory acquaintance with some of the more common aquatic organisms. While most of the groups mentioned are animals, a few other groups often confused with them are also included. Technical nomenclature is kept to a minimum.

In the use of the key, each couplet should be considered by itself, without reference to any other couplet. Select the alternative that seems most reasonable and either proceed to the couplet indicated, or else accept the name of the group as given if the selection is a terminal statement. "Cf: --" means to compare with the statement indicated. "POL" means that members of the group have been reported to be tolerant of organic pollution.

1a The body of the organisms comprising a single microscopic independent cell, or many similar and independently functioning cells associated in a colony, with little or no difference between the cells (i.e., without forming tissues); or comprising masses of multinucleate protoplasm; mostly microscopic. Single celled animals. . . . . Phylum *Protozoa*

1b The body of the organism comprising many cells of different kinds (i.e. forming tissue) may be microscopic or macroscopic. 2

2a Body of colony usually forming irregular masses or layers sometimes cylindrical, goblet-shaped, vase-shaped, elongate, serrate, or tree-like. Range from barely visible to large. 3

2b Body or colony shows some type of definite symmetry. 15

3a Colony forming irregular masses, clumps, or aggregations of material growing around, over, or clinging to surface of rocks, sticks, pipes, etc. 4

3b Irregular, or not obviously symmetrical, but essentially erect or *plant-like* individuals or colonies. 9

4a Freshwater. 8

4b Marine. 5

5a Surface of colony usually slightly rough or bristly in appearance under the microscope or hand lens. 7

5b Surface smooth. 6

5c Colony or organism erect, plant-like, branched or unbranched. 12

6a Mass very thin, usually pink or white (Coral algae). 13b

6b Mass gelatinous, either as relatively thin sheet or clumps of tongue-like club shaped projections. Transparent or colored. Star shaped groups of individual organisms often evident. Colonial tunicates or ascideans. Phylum *Chordata,* Class *Tunicata* (Cf:12b)

7a Surface webbed or bristly in appearance, stick-like crystals or "spicules" often protruding from the general surface. Pores numerous, one or more usually large and conspicuous. Sponges. .... Phylum *Porifera*

7b Colonies very thin, surface slightly roughened. Composed of minute individual cells or compartments arranged in various lacy or coral-like patterns. Each compartment inhabited by a minute bilaterally symmetrical animal which protrudes a crown of delicate tentacles. Moss animals, bryozoans.... Phylum *Bryozoa*

8a Surface of colony rough or bristly in appearance under the microscope or hand lens. Grey, green, or brown. Sponges. (Figure 1)........... Phylum *Porifera*

8b Surface colony relatively smooth, general texture of mass gelatinous, transparent. Clumps of minute individual organisms variously distributed. Moss animals, bryozoans. (Figure 2).. Phylum *Bryozoa*

9a Freshwater...................... 10

9b Marine........................... 11

10a Surface of structures rough or bristly under the microscope.............. 8a

10b Individuals or branches tubular. Usually covered with brownish cuticle to which much sediment may adhere. Moss animals, bryozoans.... Phylum *Bryozoa* (Cf·8b) (Figure 2)

11a Surface of structure appears web-like or bristly under the microscope......... 7a

11b Surface of structure an unbroken skin or cuticle, except for a limited number of functional apertures................ 13

12a Colonies minute, plant-like or coral-like. (Figure 2)....................... 13

12b Solitary, sac-like organism, either stalked or growing directly on substrate. Transparent, covered with mud or sand, or variously colored. Bilateral symmetry revealed on close investigation. Becomes flaccid when exposed and relaxed at low tide. Up to several inches in length. Seasquirts or tunicates.. Phylum *Chordata,* Class *Tunicata*

13a Compartments or apertures for separate individual animals evident........... 14

13b No evidence of the presence of individual animals. Structure profusely jointed. Individual segments hard, smooth, usually pink or white. May also grow as hard smooth thin sheet over surface of rocks. Calcareous algae or coral algae. A common Genus: .... *Corallina*

14a Minute organisms protruding from holes in colony are radially symmetrical... 17b

14b Organisms protruding are bilaterally symmetrical. (This may be assumed if they are not obviously radial) Moss animals, bryozoans. (Figure 2) (Cf:7b) .... Phylum *Bryozoa*

15a Radially symmetrical (body arranged around an axis)......................... 16

15b Fundamentally bilaterally symmetrical. 18

15c May be superficially spiral as Figure 13 35

16a Plan involves multiples of two, or else rather large numbers of tentacles around the margin of an umbrella; saucer-like, or flower-like structure............. 17

16b Body plan based on multiples of five. Outer surface hard or rough to the touch. Starfishes, brittle stars, sea urchins, sand dollars, sea cucumbers. .... Phylum *Echinodermata*

17a Jelly-like body, massive or ovoid, free swimming nearly transparent in nature. Eight rows comb-like paddle plates: Comb Jellies........ Phylum *Ctenophora*

17b Body tube-like or umbrella-like, tentacles around mouth, margin of disc, or both. Jellyfishes, corals, hydroids, sea anemones, etc... Phylum Coelenterata

18a Microscopic. Action of two ciliated lobes at anterior end in life often gives appearance of wheels. Body often segmented, accordion-like. Free swimming or attached. Rotifers or wheel animalcules (Figure 3). Phylum Trochelminthes (Rotifera)

18b Often larger, worm-like or else having strong skeleton or shell. 19

19a Skeleton (or shell) present; may be internal or external. 30

19b Body soft and/or worm-like. Skin may range from soft to parchment-like . . . 20

20a Three or more pairs of well-formed (though possibly small) jointed legs present. (Phylum Arthropoda). 36

20b Legs or appendages, if present, limited to pairs of bumps or hooks. Lobes or tentacles if present soft and fleshy, not jointed. 21

21a Body round, oval or flat in cross section 22

21b Inverted U-shaped cross section, with flat slimy undersurface or foot tapering off behind. Head with two pairs of sensitive retractile tentacles. Marine or terrestrial. Marine forms have fancy gill structures on back. Slugs. Class Gastropoda (Cf:35a) (Phylum Mollusca). 35

22a Strongly depressed or flattened. 23

22b Oval or round in cross section, or, if flat, with single sucker disc at each end. 25

23a Parasitic in bodies of higher animals. Extremely long and flat, divided into sections (like a Roman girdle). Life history may involve intermediate host. Tape worms. (Figure 5) Class Cestoda

23b Parasitic on bodies of higher animals, very muscular, strong external sucker discs (Figure 9). 29b

23c Body a single unit. Mouth and digestive system present (but no anus). 24

24a External or internal parasite on higher animals. Sucking discs present for attachment. Life history may involve two or more intermediate hosts or stages. Flukes. . . . . Class Trematoda

24b Free-living. Entire body surface covered with locomotor cilia. Eyed areas in head often appear "crossed." Pol. Free-living flatworms (Figure 6). Class Turbellaria

25a Long, slender, with snake-like motion in life. Covered with glistening cuticle. Parasitic or free-living. Microscopic to six feet in length. Round worms (Figure 7). . . . Phylum Nemathelminthes

25b Divided into sections or segments (Figures 8 and 9) 26

26a Head a more or less well-formed hard capsule with jaws, eyes, and antennae. Pol. (Figure 8) Class Insecta, (Cf:49a) . . . . Order Diptera

26b Head structure soft (except jaws, if present) (Figure 9) 27

27a Head conical or rounded, lateral appendages not conspicuous or numerous. 28

27b Head somewhat broad and blunt. Retractile jaws usually present. Soft fleshy lobes or tentacles often present in head region. Tail usually narrower. Lateral lobes or fleshy appendages on each segment. (Figure 9A) . . . . Phylum Annelida. 29

28a Minute dark colored retractile jaws present, body tapering somewhat at both ends, pairs or rings of bumps or "legs" often present, even near tail. Class Insecta. (Cf:49a) . . . . Order Diptera

28b No jaws, sides of body generally parallel except at ends, (similar to Figure 9).

Thickened area or ring usually present part way back on body. Clumps of minute bristles on most segments. .... (Phylum Annelida) 29

29a Segments with bristles, and/or fleshy lobes, or other extensions. Tube builder, borer, or burrower. Often reddish or greenish in color. Pol. Earthworms, sludge worms, clam worms, etc. .... Class Chaetopoda

29b A sucker disc at each end, the larger one posterior. External bloodsucking parasites on higher animals, often found unattached to any host. Pol. Leeches. (Figure 9B). .... Class Hirudinea

30a Skeleton internal, of true bone .... (Vertebrates) 58

30b Body covered with external skeleton or shell. (Figure 4).................. 31

31a External jointed skeleton or shell covers legs and other appendages, often leathery in nature. Jointed legged animals. (Figures 11, 12, and 14 through 29) ....(Phylum Arthropoda) 36

31b Shells limey or calcareous. (Figures 10 and 13).......................... 32

32a Marine, attached, shell conical, or purse-shaped on fleshy stalk. Crowded individuals may be more or less cylindrical. Shell composed of several parts, two of which spread apart to permit extension of "hand" for feeding. Barnacles. Phylum Arthropoda, Class Cirripedia

32b Shells one or two, or several arranged as transverse plates................ 33

33a Half inch or less in length. Two clam-like shells. Soft parts inside include delicate flattened jointed appendages. Phyllopods or branchipods (Figures 11 and 12) Phylum Arthropoda, Class Crustacea, Subclass Branchipoda (Cf: 39a)

33b Soft parts covered with thin skin, often slimy............ (Phylum Mollusca) 34

34a Marine, oval in shape, eight shells transversely jointed. Chitons. .... Class Amphineura

34b Shells one or two in number. Snails, clams, etc. (Figures 10-13)........ 35

35a Shell, single may be a spiral, a flattened cone, degenerate, or missing. Pol. Snails and slugs..... Class Gastropoda

35b Shell, double, right and left halves, hinged at one point. Mussels, clams, oysters. .... Class Pelecypoda

36a Head, thorax, and abdominal body regions usually distinct. Three pairs of regular walking legs, or their rudiments. Wings present in all adults and rudiments in some larvae. Leglike appendages or gills present on abdomens of some larvae. No marine forms. (Phylum Arthropoda, ...Class Insecta) 47

36b More than three pairs of legs apparently present. (Figures 14-19) 37

37a Body elongated, head broad and flat with strong jaws. Appendages following first three pairs of legs are rounded tapering filaments. Up to 3 inches long. Dobson fly and fish fly larvae. Class Insecta, ... Order Megaloptera

37b Four or more pairs of true legs..... 38

38a Four pairs of legs. Body rounded, bulbous, head minute. Often brown or red. Water mites. (Figure 15) Phylum Arthropoda, Class Arachnida, Order Acari

38b Five or more pairs of locomotor appendages (walking or swimming legs) gills, two pairs of atenna. (Figures 16-19, 11-12) Crustaceans. Phylum Arthropoda, Class Crustacea.................. 39

39a Ten or more pairs of flattened, leaf-like swimming (and respiratory) appendages. Many species swim constantly in life, some normally upside down. (Figure 16) Fairy shrimps, phyllopods, or branchipods. .... Subclass Branchipoda

39b Less than ten pairs of locomotor appendages...................... 40

40a Body and legs enclosed in bivalved (2 halves) shell which may or may not completely hide them. (Figures 11-12) 41

40b Appendages more rounded than leaf-like in cross section, or if appendages are flattened for swimming, eyes are on stalks (pelagic shrimps). May be large or minute......................... 43

41a One pair of branched antennae enlarged for locomotion, extend outside of shell (carapace). Single eye usually visible. Cyclops or cladocera. Subclass Cladocera

41b Locomotion accomplished by body legs (not antennae). (Figure 16).......... 42

42a Appendages leaf-like, flattened, more than ten pairs. (Branchipods)....... 39a

42b Animal less than three mm in length. Appendages more or less slender and jointed, often used for walking. (Figure 11) Shells opaque. Ostracodes. .... Subclass Ostracoda

43a Body a series of six or more essentially similar segments, differing mainly in size..................................... 44

43b Front part of body enlarged into a somewhat separate body unit (cephalothorax) often covered with a single piece of shell (carapace). Back part (abdomen) may be relatively small, even folded underneath front part. (Figure 19)............. 45

44a Body compressed laterally, i.e., high and thin. Scuds, amphipods. (Figure 17) .... Subclass Amphipoda

44b Body depressed, i.e., low and broad. Flat gills contained in chamber beneath tail. (Figure 18) Sowbugs. .... Subclass Isopoda

45a Minute in size. Often somewhat elongated and cylindrical, with abdomen extending straight out behind ending in two small projections. Two relatively enormous masses of eggs are often attached to female. Locomotion by means of two enlarged, unbranched antennae, the only large appendages on the body. (Figure 19) Copepods............. Subclass Copepoda

45b Larger. Carapace (shell of cephalothorax, the enlarged front section) usually more conspicuous. 5 pairs of walking legs including claws, if present. Appendages present on abdomen................ 46

46a Eyes immovable, in broad shell shaped like a horseshoe, a mud dweller. Long sharp pointed triangular tail extends out behind. Flat "book" gills beneath abdomen. Horseshoe crab. Subclass Arachnoidea, ... Genus Limulus

46b Eyes on movable stalks. Abdomen tightly folded back under cephalothorax (crabs) or continued out behind thorax to end in an expanded "flipper" or swimming paddle. Crabs, shrimps, crayfishes, lobsters, etc................. Subclass Decapoda

47a Two pairs of functional wings (one pair may be more or less hardened as protection for the other pair). Adult insects which normally live on or in the water (Figures 22, 25, 28, 29) 57

47b No functional wings, though "pads" in which they are developing may be visible. Some resemble adults very closely, others differ extremely (Figures 8, 23, 24, 26, 27) 48

48a External pads or cases in which wings develop clearly visible.............. 53

48b More or less worm-like, no external evidence of wing development........ 49

49a No jointed legs (sucker discs, hooks, prolegs, breathing tubes, and other such structures may be present. (Figures 8, 9D) Larvae of two winged flies, midges, etc. Pol.................... Order Diptera

49b Three pairs of jointed thoracic legs, head capsule well-formed................ 50

50a Minute (2 - 4mm), living on surface film. Tail a strong organ that can be hooked into a "catch" beneath thorax. When released, animal jumps into the air. Not usually classed as aquatic. These are adults, no wings are ever grown. (Figure 20) Springtails..........Order Collembola

50b Larger (rarely collected under 5 mm), worm-like, living beneath the surface. 51

51a Construct fixed or movable cases of bottom materials. (Figure 21) Abdominal segments often with minute gill filaments, no long lateral filaments. Generally cylindric in shape. Caddisfly larvae. ....Order Trichoptera

51b Free living, i.e., not case-building. 52

52a Somewhat flattened and relatively massive in appearance. First segment of thorax about same size as head, following two segments smaller. Each abdominal segment with rather stout, tapering lateral filament about as long as body is wide. (Figure 14) Dobsonfly, alderfly, and fishfly larvae. Order Megaloptera

52b Generally rounded in cross section. Lateral filaments if present tend to be longer and thinner. (Figure 23) (A few forms extremely flattened, like a suction cup: Psephenidae). Beetle larvae. .... Order Coleoptera

53a Two or three filaments or other structures extending out from end of abdomen. (Figures 24B, 24D, 26, 27)........ 55

53b Abdomen ending abruptly, unless terminal segment itself is extended as single structure. (Figure 24C)........... 54

54a Head broad, eyes widely spaced. Front of face covered by extensible folded mouthparts often called a "mask." Larvae of dragonflies or darning needles. Pol. (Figure 24E)........... Order Odonata ....(Suborder Anisoptera)

54b Mouthparts for piercing and sucking. Body form varies extremely. Water bugs, walking sticks, water boatmen, backswimmers, electric light bugs, water striders, and others. (Figure 25) .... Order Hemiptera

55a Tail extensions (caudal filaments) two. Stonefly larvae. (Figure 26) .... Order Plecoptera

55b Tail extensions usually three. (Figures 24B, 24D, 27)........................... 56

56a Tail extensions long and slender. Rows of hairs may give them a feather-like appearance. Larvae of mayflies. .... Order Ephemeroptera

56b Tail extensions flat, elongated plates. Head broad with widely spaced eyes, abdomen relatively long and slender. Larvae of damselflies. Order Odonata ....(Suborder Zygoptera)

57a External wings or wing covers form a hard protective dome over the inner wings folded beneath, and the abdomen. Beetles. (Figure 28).........Order Coleoptera

57b External wings somewhat membranous, usually relatively flat as compared to wing covers of beetles. Mouthparts for piercing and sucking. Body form various, (Cf:53b). True bugs. (Figure 25) Order Hemiptera

58a Appendages present in pairs (fins, legs, wings)............................ 60

58b Mouth a round suction disc. No paired appendages....................... 59

59a Body long and slender. Several holes alongside of head. Lampreys and hagfishes. Sub-phylum Vertebrata. Class Cyclostomata

59b Body plump, oval, tail extending out abruptly. Larvae of frogs and toads. Legs appear one at a time during metamorphosis to adult form. Tadpoles...Class Amphibia

60a Paired appendages are legs..........62

60b Paired appendages are fins.......... 61

61a Gills covered with a flap or "operculum." True fishes.............Class Pisces

61b Gill chambers open directly to the outside. Skin often rough and scratchy or penetrated by clumps of short sharp spines. Essentially marine. Sharks, skates, and rays. .... Class Elasmobranchii

62a Digits with claws, nails, or hoofs.... 63

62b Skin naked, no claws on digits. Frogs, toads, and salamanders. Class Amphibia

63a Warm blooded..................... 64

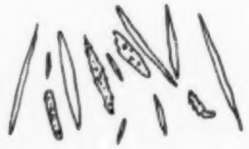
63b Body covered with horny scales or plates. Reptiles..................Class Reptilia

64a Body covered with feathers. Birds. ....Class Aves

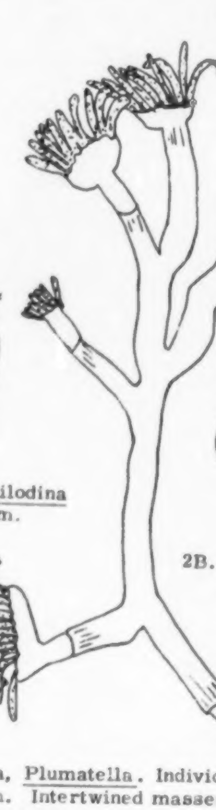
64b Body covered with hair. Mammals. ....Class Mammalia

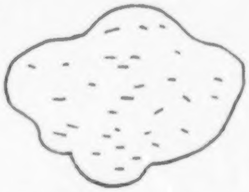
APPENDIX A
LIST OF INCLUDED GROUPS

Phyla or Subphyla	Classes or Subphyla	Classes or Orders	Other
Protozoa	-----		
Porifera	-----		
Coelenterata	-----		
Platyhelminthes	Turbellaria		
	Trematoda		
	Cestoda		
Rotifera	-----		
Nemathelminthes	-----		
Bryozoa	-----		
Annelida	Chaetopoda		
	Hirudinea		
Arthropoda	Crustacea	Branchiopoda	
		Cladocera	
		Ostracoda	
		Amphipoda	
		Isopoda	
		Copepoda	
		Decapoda	
		Cirripedia	
	Arachnoidea	Acari	
		Xiphosura	Limulus
	Insecta	Collembola	
		Plecoptera	
		Ephemeroptera	
		Odonata	
		Hemiptera	
		Megaloptera	
		Trichoptera	
		Coleoptera	
		Diptera	
Mollusca	Gastropoda		
	Pelecypoda		
Enchinodermata	-----		
Chordata	Tunicata		
	Cyclostomata		
	Pisces		
	Amphibia		
	Reptilia		
	Aves		
	Mammalia		
Algae	-----		Corallina

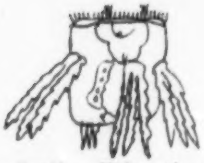


1. Spongilla spicules
Up to .2 mm. long.

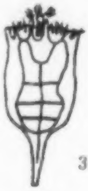




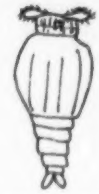
2B. Bryozoal mass. Up to several feet diam.



3A. Rotifer, Polyarthra
Up to .3 mm.

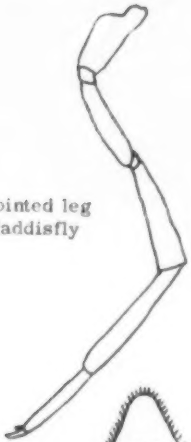


3B. Rotifer, Keratella
Up to .3 mm.

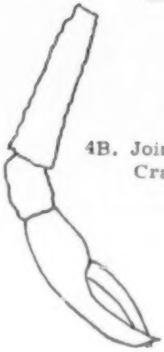


3C. Rotifer, Philodina
Up to .4 mm.

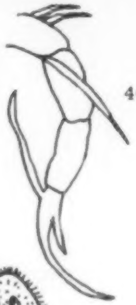
2A. Bryozoa, Plumatella. Individuals up to 2 mm. Intertwined masses may be very extensive.



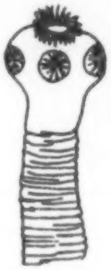
4A. Jointed leg
Caddisfly



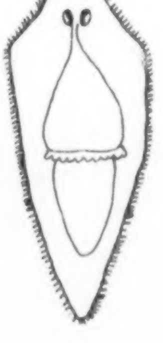
4B. Jointed leg
Crayfish



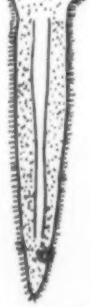
4C. Jointed leg
Ostracod



5. Tapeworm head, Taenia. Up to 25 yds. long

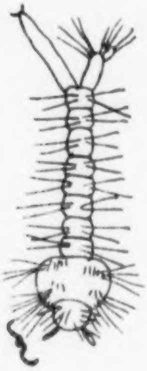


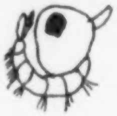
Turbellaria, Mesostoma
p to 1 cm.



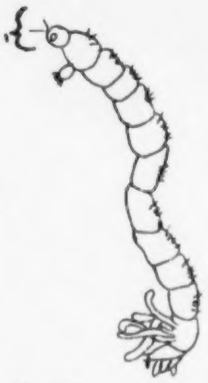
6B. Turbellaria, Dugesia
Up to 1.6 cm.

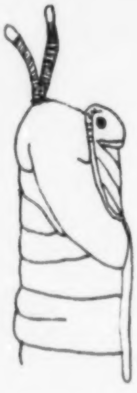
7. Nematodes. Free living forms commonly up to 1 mm., occasionally more.





8B. Diptera, Mosquito pupa. Up to 5 mm.



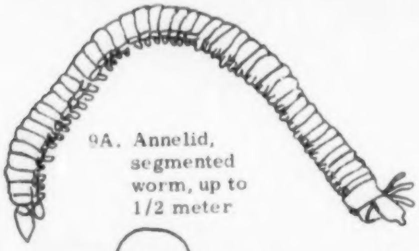


2

8A. Diptera, Mosquito larvae Up to 15 mm. long.

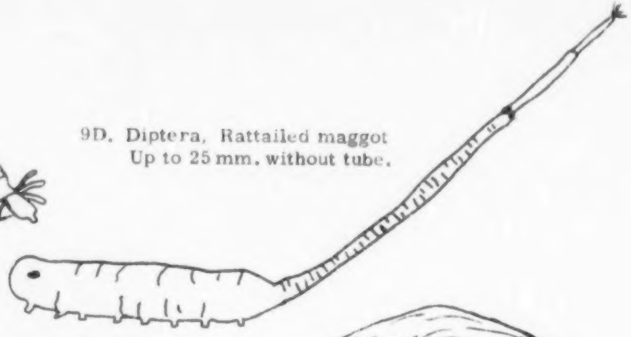
8C. Diptera, Tendiped larvae. Up to 2 cm.

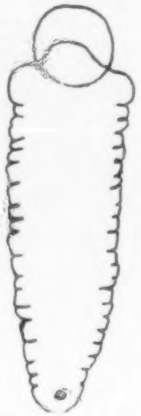
8E. Diptera, crane fly pupa. Up to 2.5 cm.

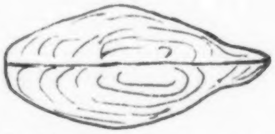


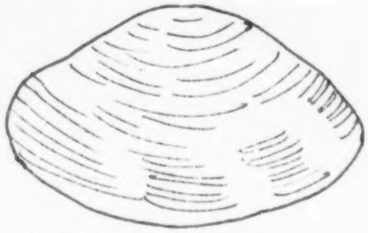
9A. Annelid, segmented worm, up to 1/2 meter

9D. Diptera, Rattailed maggot Up to 25 mm. without tube.

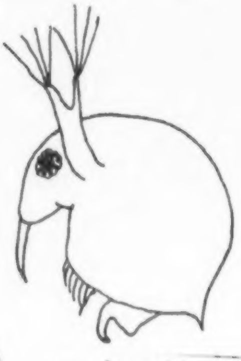






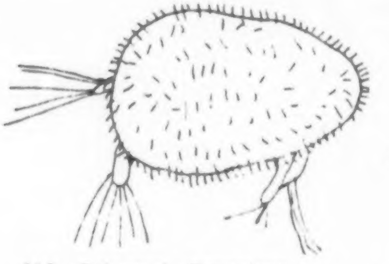


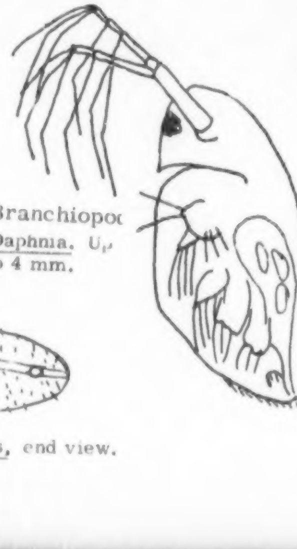
10B. Alasmidonta, end view.



10A. Pelecyopod, Alasmidonta Side view, up to 18 cm. long.

9B. Annelid, leech up to 20 cm.

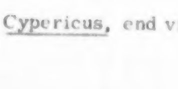




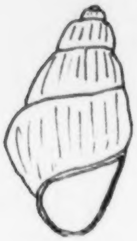
12A. Branchiopo Daphnia. Up to 4 mm.

12B. ranchiopod Bosmina. Up to 2 mm.

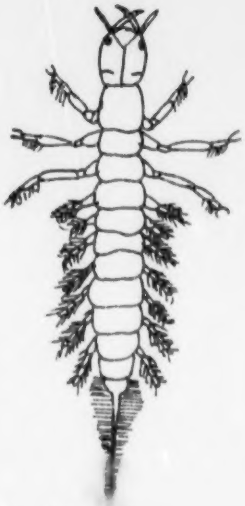
11A. Ostracod, Cypericus Side view, up to 7 mm.



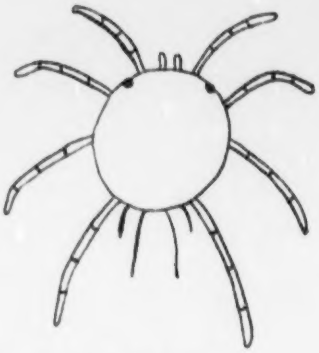
11B. Cypericus, end view.



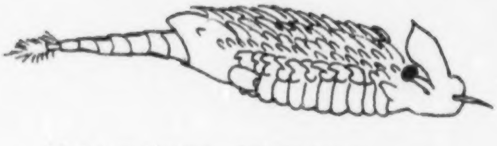
13. Gastropod, Viviparus
Up to 3 inches.



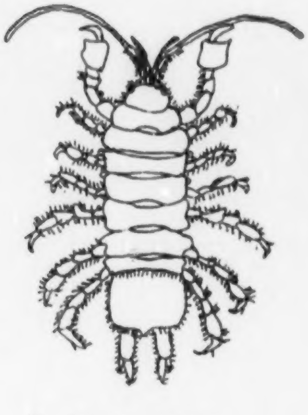
14. Megaloptera,
Alderfly larvae
Up to 25 mm.



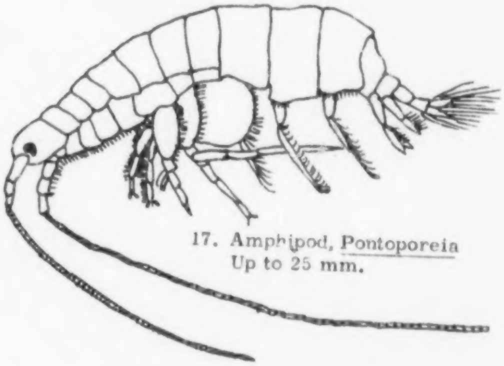
15. Water mite,
Up to 3 mm.



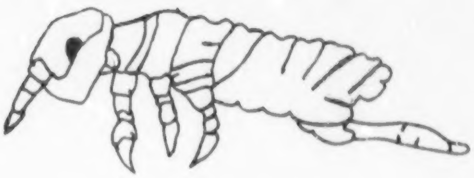
16. Fairy Shrimp, Eubranchipus
Up to 5 cm.



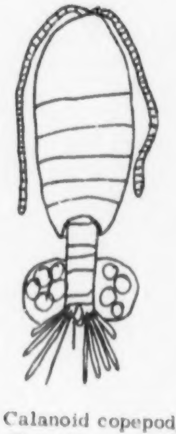
18. Isopod, Asellus
Up to 25 mm.



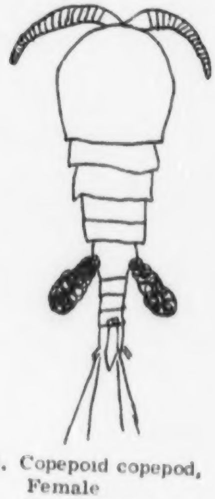
17. Amphipod, Pontoporeia
Up to 25 mm.



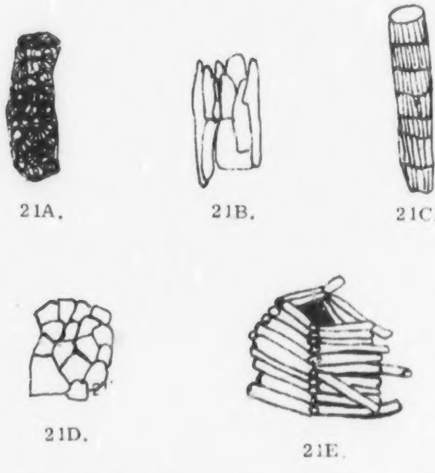
20. Collembola, Podura
Up to 2 mm. long



19A. Calanoid copepod,
Female
Up to 3 mm.

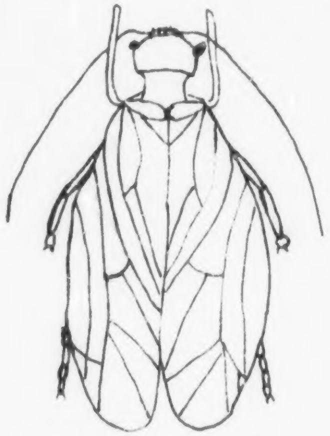


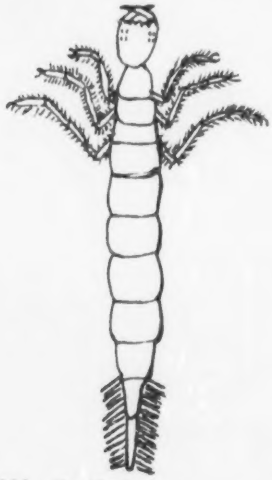
19B. Copepoid copepod,
Female
Up to 25 mm.

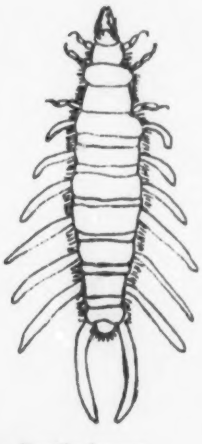

21A. 21B. 21C.

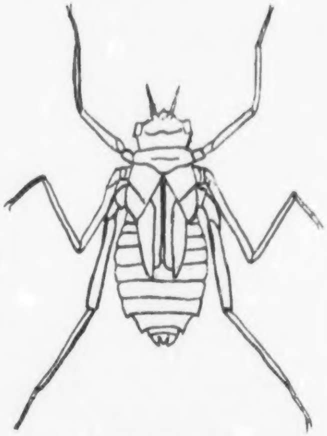
21D. 21E.

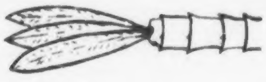
21. Tricoptera, Larvae cases, mostly 1-2 cm.

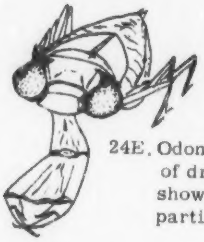

22. Megaloptera, alderfly Up to 2 cm.

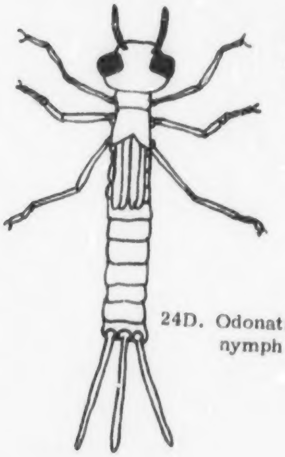

23A. Beetle larvae, Dytisidae, Usually about ½ cm.

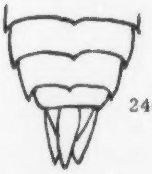

23B. Beetle larvae, Hydrophilidae, Usually about 1 cm.

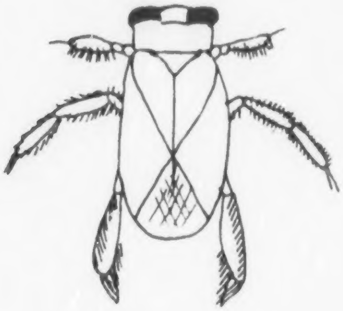

24A. Odonata, dragonfly nymph up to 3 or 4 cm.


24B. Odonata, tail of damselfly nymph (side view)

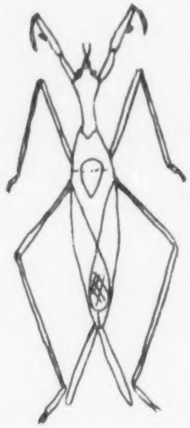

24E. Odonata, front view of dragonfly nymph showing "mask" partially extended


24D. Odonata, damselfly nymph (top view)

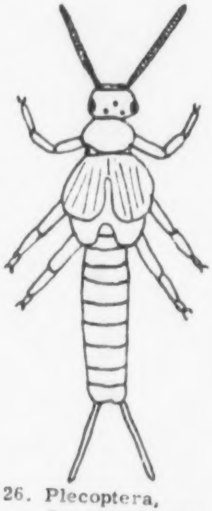

24C. Odonata, tail of dragonfly nymph (top v.



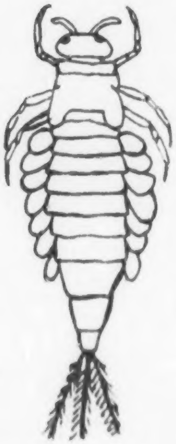
25A. Hemiptera,
Water Boatman
About 1 cm.



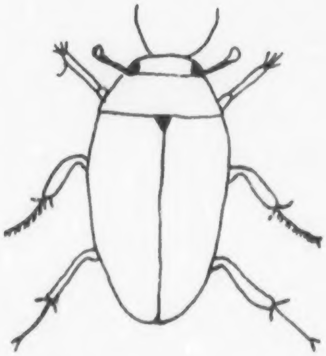
25B. Hemiptera,
Water Scorpion
About 4 cm.



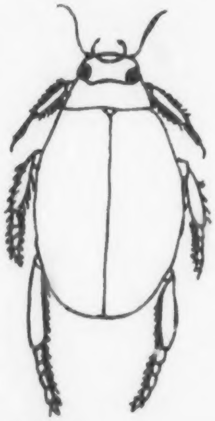
26. Plecoptera,
Stonefly nymph
Up to ½ cm.



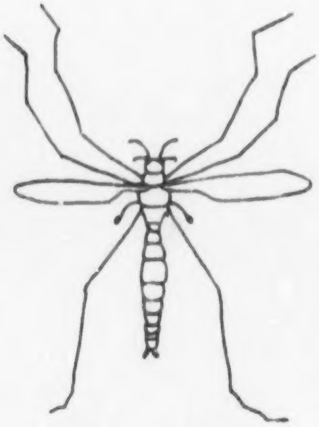
27.Ephemeroptera,
Mayfly nymph
Up to ½ cm.



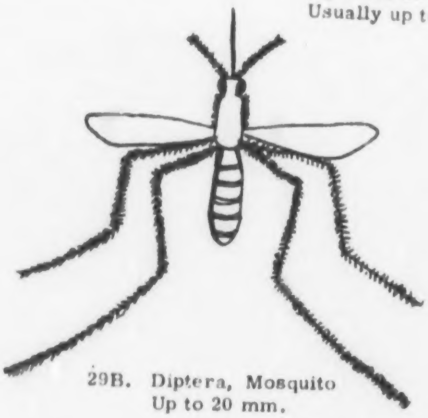
28A. Coleoptera,
Water scavenger
beetle. Up to 4 cm.



28B. Coleoptera,
Dytiscid beetle
Usually up to 2½ cm.



[illegible]. Diptera, Crane
fly. Up to 2½ cm.



29B. Diptera, Mosquito
Up to 20 mm.

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Descriptors:
Aquatic Life; Systematics

USING BENTHIC BIOTA IN WATER QUALITY EVALUATIONS

I BENTHOS ARE ORGANISMS GROWING ON OR ASSOCIATED PRINCIPALLY WITH THE BOTTOM OF WATERWAYS

Benthos is the noun.

Benthonic, benthal and benthic are adjectives.

II THE BENTHIC COMMUNITY

A Composed of a wide variety of life forms that are related because they occupy "common ground"--the waterways bottom substrates. Usually they are attached or have relatively weak powers of locomotion. These life forms are:

1 Bacteria

A wide variety of decomposers work on organic materials, breaking them down to elemental or simple compounds (heterotrophic). Other forms grow on basic nutrient compounds or form more complex chemical compounds (autotrophic).

2 Algae

Photosynthetic plants having no true roots, stems, and leaves. The basic producers of food that nurtures the animal components of the community.

3 Flowering Aquatic Plants (Pondweeds)

The largest flora, composed of complex and differentiated tissues. Many are rooted.

4 Microfauna

Animals that pass through a U.S. Standard Series No. 30 sieve, but are retained on a No. 100 sieve. Examples are rotifers and microcrustaceans. Some forms have organs for attachment to substrates, while others burrow into soft materials or occupy the interstices between rocks, floral or faunal materials.

5 Meiofauna

Animals, mostly metazoans, that can pass a 1.0 mm to 0.5 mm screen. Examples are naiad worms and flatworms.

6 Macrofauna

Animals that are retained on a No. 30 sieve. This group includes the insects, worms, molluscs, and occasionally fish. Fish are not normally considered as benthos, though there are bottom dwellers such as sculpins and darters.

B It is a self-contained community, though there is interchange with other communities. For example: Plankton settles to it, fish prey on it and lay their eggs there, terrestrial detritus leaves are added to it, and many aquatic insects migrate from it to the terrestrial environment for their mating cycles.

C It is a stationary water quality monitor. The low motility of the biotic components requires that they "live with" the quality changes of the over-passing waters. Changes imposed in the long-lived components remain visible for extended periods, even after the cause has been eliminated. Only time will allow a cure for the community by drift and reproduction.

III HISTORY OF BENTHIC OBSERVATIONS

A Ancient literature records the vermin associated with fouled waters.

B 500-year-old fishing literature refers to animal forms that are fish food and used as bait.

C The scientific literature associating biota to water pollution problems is over 100 years old (Mackenthun and Ingram, 1964).

D Early this century, applied biological investigations were initiated.

  1 The entrance of State boards of Health into water pollution control activities.

  2 Creation of state conservation agencies.

  3 Industrialization and urbanization.

  4 Growth of limnological programs at universities.

E A decided increase in benthic studies occurred in the 1950 decade, and much of today's activities are strongly influenced by developmental work conducted during this period. Some of the reasons for this are:

  1 Movement of the universities from "academic biology" to applied pollution programs.

  2 Entrance of the federal government into enforcement aspects of water pollution control.

  3 A rising economy and the development of federal grant systems.

  4 Environmental Protection Programs are a current stimulus.

IV WHY THE BENTHOS?

A It is a natural monitor

B The community contains all of the components of an ecosystem.

  1 Reducers

  2 Producers

  3 Consumers

    a Detritivores and bacterial feeders

    b Herbivores

    c Predators

C Economy of Survey

  1 Manpower

  2 Time

  3 Equipment

D Extensive Supporting Literature

E Advantages of the Macrobenthos

  1 Relatively sessile

  2 Life history length

  3 Fish food organisms

  4 Reliability of Sampling

  5 Dollars/information

  6 Predictability

  7 Universality

V REACTIONS OF THE COMMUNITY TO POLLUTANTS

A Destruction of Organism Types

  1 Beginning with the most sensitive forms, pollutants kill in order of sensitivity until the most tolerant form is the last survivor. This results in a reduction of variety or diversity of organisms.

  2 The usual order of macroinvertebrate disappearance on a sensitivity scale below pollution sources is shown in Figure 2.

Water Quality Deteriorating ↓	Stoneflies Mayflies Caddisflies Amphipods Isopods Midges Oligochaetes	↑ Water Quality Improving

As water quality improves, these reappear in the same order.

B The Number of Survivors Increase

1 Competition and predation are reduced between forms.

2 When the pollutant is a food (plants, fertilizers, animals, organic materials)

C The Number of Survivors Decrease

1 The material added is toxic or has no food value.

2 The material added produces toxic conditions as a byproduct of decomposition (e.g., large organic loadings produce an anaerobic environment resulting in the production of toxic sulfides, methanes, etc.)

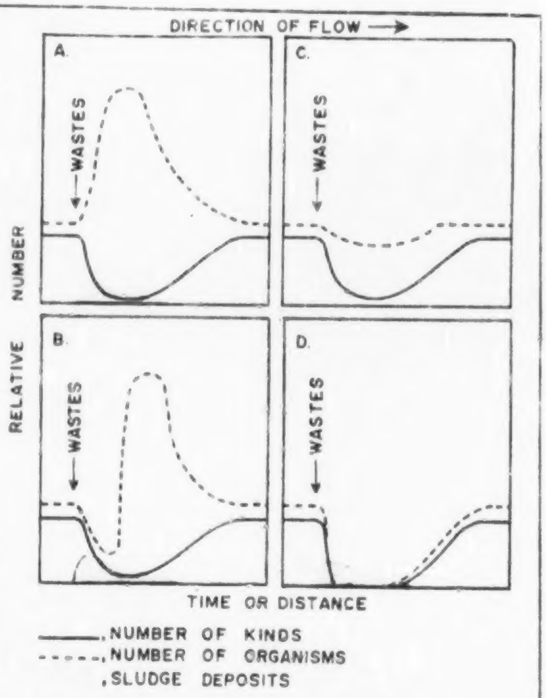
D The Effects May be Manifest in Combinations

1 Of pollutants and their effects.

2 Vary with longitudinal distribution in a stream. (Figure 1)

E Tolerance to Enrichment Grouping (Figure 2)

Flexibility must be maintained in the establishment of tolerance lists based on the response of organisms to the environment because of complex relationships among varying environmental conditions. Some general tolerance patterns can be established. Stonefly nymphs, mayfly naiads, hellgrammites, and caddisfly larvae represent a grouping (sensitive or intolerant) that is generalized quite sensitive to environmental changes. Blackfly larvae, scuds, sowbugs, snails, fingernail clams, dragonfly nymphs, damselfly nymphs, and most kinds of midge larve are intermediate (facultative or intermediate) in tolerance. Sludge-worms, some kinds of midge larvae (bloodworms), and some leeches are tolerant to comparatively heavy loads of organic pollutants. Sewage mosquitoes and rat-tailed maggots are tolerant of anaerobic environments.



Four basic responses of bottom animals to pollution. A. Organic wastes eliminate the sensitive bottom animals and provide food in the form of sludges for the surviving tolerant forms. B. Large quantities of decomposing organic wastes eliminate sensitive bottom animals and the excessive quantities of byproducts of organic decomposition inhibit the tolerant forms: in time, with natural stream purification, water quality improves so that the tolerant forms can flourish, utilizing the sludges as food. C. Toxic materials eliminate the sensitive bottom animals: sludge is absent and food is restricted to that naturally occurring in the stream, which limits the number of tolerant surviving forms. Very toxic materials may eliminate all organisms below a waste source. D. Organic sludges with toxic materials reduce the number of kinds by eliminating sensitive forms. Tolerant survivors do not utilize the organic sludges because the toxicity restricts their growth.

Figure 1

F Structural Limitations

1 The morphological structure of a species limits the type of environment it may occupy.

a Species with complex appendages and exposed complicated respiratory structures, such as stonefly nymphs, mayfly nymphs, and caddisfly larvae, that are subjected to a constant deluge of setteable particulate matter soon abandon the polluted area because of the constant preening required to maintain mobility or respirotory functions; otherwise, they are soon smothered.

b Benthic animals in depositing zones may also be burdened by "sewage fungus" growths including stalked protozoans. Many of these stalked protozoans are host specific.

2 Species without complicated external structures, such as bloodworms and sludgeworms, are not so limited in adaptability.

a A sludgeworm, for example, can burrow in a deludge of particulate organic matter and flourish on the abundance of "manna."

b Morphology also determines the species that are found in riffles, on vegetation, on the bottom of pools, or in bottom deposits.

VI SAMPLING PROCEDURES

A Fauna

1 Qualitative sampling determines the variety of species occupying an area. Samples may be taken by any method that will capture representatives of the species present. Collections from such samplings indicate changes in the environment, but generally do not accurately reflect the degree of change. Mayflies, for example, may be reduced from 100 to 1 per square foot. Qualitative data would indicate the presence of both species, but might not necessarily delineate the change in predominance from mayflies to sludgeworms. The stop net or kick sampling technique is often used.

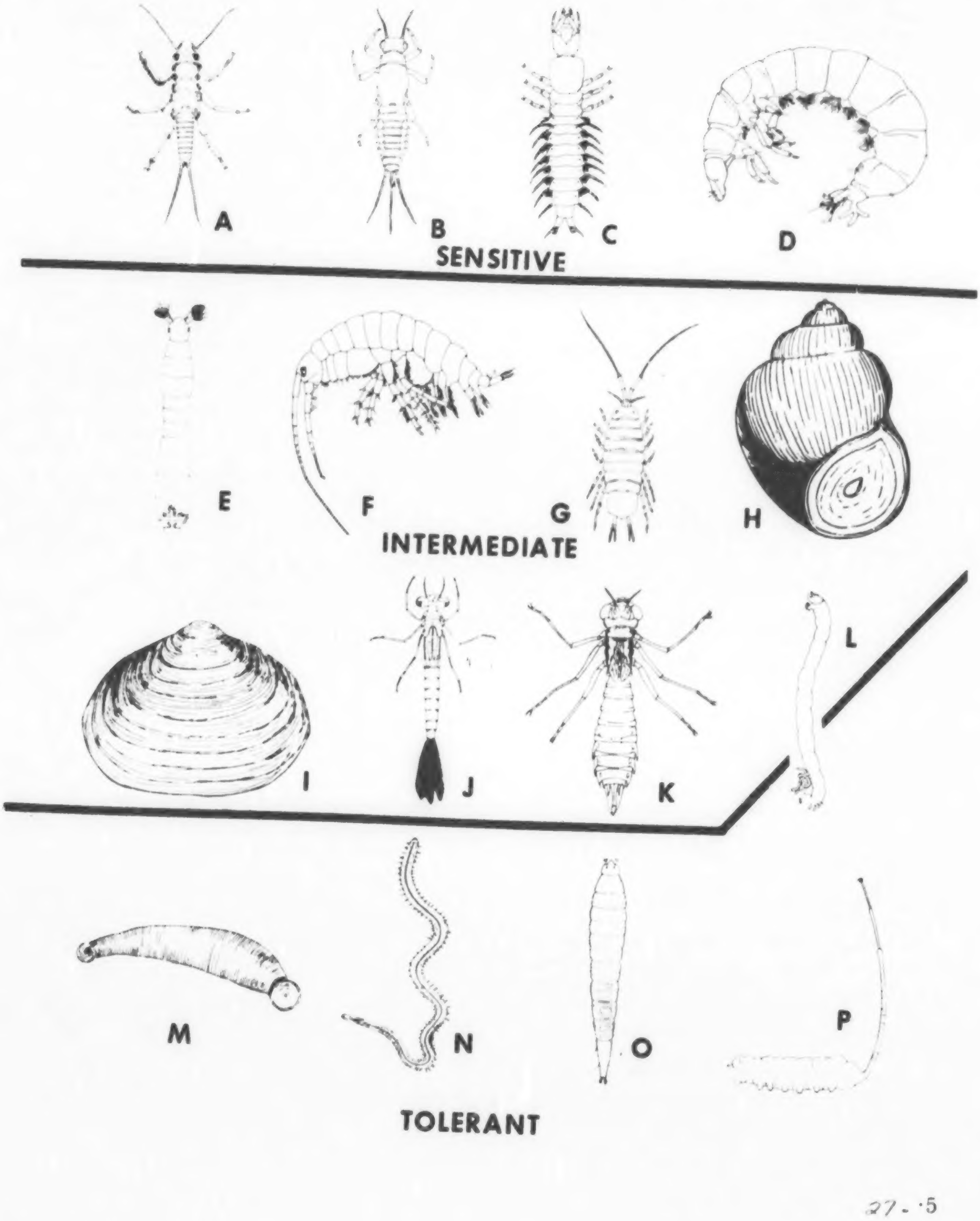
2 Quantitative sampling is performed to observe changes in predominance. The most common quantitative sampling tools are the Petersen and Elkman

REPRESENTATIVE BOTTOM-DWELLING MACROANIMALS

Drawings from Geckler, J., K. M. Mackenthun and W. M. Ingram, 1963, Glossary of Commonly Used Biological and Related Terms in Water and Waste Water Control, DHEW, PHS, Cincinnati, Ohio, Pub. No. 999-WP-2.

A	Stonefly nymph	(Plecoptera)
B	Mayfly nymph	(Ephemeroptera)
C	Hellgrammite or Dobsonfly larvae	(Megaloptera)
D	Caddisfly larvae	(Trichoptera)
E	Black fly larvae	(Simuliidae)
F	Scud	(Amphipoda)
G	Aquatic sowbug	(Isopoda)
H	Snail	(Gastropoda)
I	Fingernail clam	(Sphaeriidae)
J	Damselfly nymph	(Zygoptera)
K	Dragonfly nymph	(Anisoptera)
L	Bloodworm or midge fly larvae	(Chironomidae)
M	Leech	(Hirudinea)
N	Sludgeworm	(Tubificidae)
O	Sewage fly larvae	(Psychodidae)
P	Rat-tailed maggot	(Tubifera-Eristalis)

KEY TO FIGURE 2


A
B
C
D
SENSITIVE
E
F
G
H
INTERMEDIATE
I
J
K
L
M
N
O
P
TOLERANT

grabs and the Surber stream bottom or square-foot sampler. Of these, the Petersen grab samples the widest variety of substrates. The Ekman grab is limited to fine-textured and soft substrates, such as silt and sludge.

The Surber sampler is designed for sampling riffle areas; it requires moving water to transport dislodged organisms into its net and is limited to depths of two feet or less.

3 The collected sample is screened with a standard sieve to concentrate the organisms; these are sorted from the retained material, and the number of each kind determined. Data are then adjusted to number per unit area, usually to number of bottom per square meter.

4 Independently, neither qualitative not quantitative data suffice for thorough analyses of environmental conditions. A cursory examination to detect damage may be made with either method, but a combination of the two gives a more precise determination. If a choice must be made, quantitative sampling would be best, because it incorporates a partial qualitative sample.

B Flora

1 Direct quantitative sampling of naturally growing bottom algae is difficult. It is basically one of collecting algae from a standardor uniform area of the bottom substrates without disturbing the delicate growths and thereby distort the sample. Indirect quantitative sampling is the best available method.

Artificial substrates, such as wood blocks, glass or plexiglass slides, bricks, etc., are placed in a stream. Bottom-attached algae will grow on these artificial substrates. After two or more weeks, the artificial substrates are removed for analysis. Algal growths are scraped from the substrates and the qunatity measured. Since the exposed substrate area and exposure periods are equal at all of the sampling sites, differences in the quantity of algae can be related to changes in the quality of water flowing over the substrates.

2 The quantity of algae on artificial substrates can be measured in several ways. Microscopic counts of algal cells and dry weight of algal material are long established methods.

Microscopic counts involve thorough scraping, mixing, and suspension of the algal cells. From this mixture an aliquot of cells is withdrawn for enumeration under a microscope. Dry weight is determined by drying and weighing the algal sample, then igniting the sample to burn off the algal materials, leaving inert inorganic materials that are again weighed. The difference between initial weight and weight after ignition is attributed to algae.

Any organic sediments, however, that settle on the artificial substrate along with the algae are processed also. Thus, if organic wastes are present appreciable errors may enter into this method.

3 During the past decade, chlorophyll analysis has become a popular method for estimating algal growth. Chlorophyll is extracted from the algae and is used as an index of the quantity of algae present. The advantages of chlorophyll analysis are rapidity, simplicity, and vivid pictorial results.

The algae are scrubbed from the artificial substrate samples, ground, then each sample is steeped in equal volumes, 90% aqueous acetone, which extracts the chlorophyll from the algal cells. The chlorophyll extracts may be compared visually.

Because the chlorophyll extracts fade with time, colorimentry should be used for permanent records. For routine records, simple colorimeters will suffice. At very high chlorophyll densities, interference with colorimetry occurs, which must be corrected through serial dilution of the sample or with a nomograph.

4 Autotrophic Index

The chlorophyll content of the periphyton is used to estimate the algal biomass and as an indicator of the nutrient content (or trophic status) or toxicity of the water and the taxonomic composition of the community. Periphyton growing in surface water relatively free of organic pollution consists largely of algae, which contain approximately 1 to 2 percent chlorophyll a by dry weight. If dissolved or particulate organic matter is present in high concentrations, large populations of filamentous bacteria, stalked protozoa, and other nonchlorophyll bearing microorganisms develop and the percentage of chlorophyll a is then reduced. If the biomass-chlorophyll a relationship is expressed as a ratio (the autotrophic index), values greater than 100 may result from organic pollution (Weber and McFarland, 1969; Weber, 1973).

$$\text{Autotrophic Index} = \frac{\text{Ash-free Wgt (mg/m}^2\text{)}}{\text{Chlorophyll a (mg/m}^2\text{)}}$$

VII FACTORS INVOLVED IN DATA INTERPRETATION

Two very important factors in data evaluation are a thorough knowledge of conditions under which the data were collected and a critical assessment of the reliability of the data's representation of the situation.

A Maximum-Minimum Values

The evaluation of physical and chemical data to determine their effects on aquatic organisms is primarily dependent on maximum and minimum observed values. The mean is useful only when the data are relatively uniform. The minimum or maximum values usually create acute conditions in the environment.

B Identification

Precise identification of organisms to species requires a specialist in limited taxonomic groups. Many immature aquatic forms have not been associated with the adult species. Therefore, one who is certain of the genus but not the species should utilize the generic name, not a potentially incorrect species name. The method of interpreting biological data on the basis of numbers of kinds and numbers of organisms will typically suffice.

C Lake and Stream Influence

Physical characteristics of a body of water also affect animal populations. Lakes or impounded bodies of water support different faunal associations from rivers. The number of kinds present in a lake may be less than that found in a stream because of a more unifrom habitat. A lake is all pool, but a river is composed of both pools and riffles. The nonflowing water of lake exhibits a more complete settling of particulate organic matter that naturally supports a higher population of detritus consumers. For these reasons, the bottom fauna of a lake or impoundment cannot be directly compared with that of a flowing stream.

D Extrapolation

How can bottom-dwelling macrofauna data be extrapolated to other environmental components? It must be borne in mind that a component of the total environment is being sampled. If the sampled component exhibits changes, then so must the other interdependent components of the environment. For example, a clean stream with a wide variety of desirable bottom organisms would be expected to have a wide variety of desirable bottom fishes; when pollution reduces the number of bottom organisms, a comparable reduction would be expected in the number of fishes. Moreover, it would be logical to conclude that any factor that eliminates all bottom organisms would eliminate most other aquatic forms of life.

VII IMPORTANT ASSOCIATED ANALYSES

A The Chemical Environment

1 Dissolved oxygen

2 Nutrients

3 Toxic materials

4 Acidity and alkalinity

5 Etc.

B The Physical Environment

1 Suspended solids

2 Temperature

3 Light penetration

4 Sediment composition

5 Etc.

IX AREAS IN WHICH BENTHIC STUDIES CAN BEST BE APPLIED

A Damage Assessment

If a stream is suffering from pollutants, the biota will so indicate. A biologist can determine damages by looking at the "critter" assemblage in a matter of hours. Usually, if damages are not found, it will not be necessary to alert the remainder of the agency's staff, pack all the equipment, pay travel and per diem, and then wait five days before enough data can be assembled to begin evaluation.

B By determining what damages have been done, the potential cause "list" can be reduced to a few items for emphasis and the entire "wonderful worlds" of science and engineering need not be practiced with the result that much data are discarded later because they were not applicable to the problem being investigated.

C Good benthic data associated with chemical, physical, and engineering can be data used to predict the direction of future changes and to estimate the amount of pollutants that need to be removed from the waterways.

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This outline was prepared by Lowell E. Keup, Chief, Technical Studies Branch, Div. of Technical Support, EPA, Washington, D. C. 20242, and revised by Ralph M. Sinclair, Aquatic Biologist, National Training Center, EPA, WPO, Cincinnati, OH 45268.

Descriptors:
Aquatic Life, Benthos, Water Quality, Degradation, Environmental Effects, Trophic Level, Biological Communities, Ecological Distributor

BIOLOGICAL FIELD METHODS

I INTRODUCTION

A Due to the nature of ecological interrelationships, methods for the collection of different types of aquatic organisms differ. In general we can recognize those that swim or float and those that crawl, those that are big and those that are little. Each comprises a part of "the life" at any given survey station and consequently a "complete" collection would include all types.

B Field methods in the following outline are grouped under four general categories, the collection of:

1 Benthos (or bottom dwelling organisms). These may be attached, crawling, or burrowing forms.

2 Plankton (plancton). These are all of the microscopic plants and animals normally swimming or suspended in the open water.

3 Periphyton or "aufwuchs". This is the community of organisms associated with the surfaces of objects. Some are attached, some crawl. The group is intermediate between the benthos and the plankton.

4 Nekton. Nekton are the larger, free swimming active animals such as shrimp or fishes.

C Aquatic mammals and birds, in most cases, require still other approaches and are not included.

D There is little basic difference between biological methods for oceanic, estuarine, or freshwater situations except those dictated by the physical nature of the environments and the relative sizes of the organisms. Fish, benthos, and plankton collection is essentially the same whether conducted in Lake Michigan, Jones' Beach, or the Sargasso Sea.

1 Marine organisms range to larger sizes, and the corrosive nature of seawater dictates special care in the design and maintenance of marine equipment. Site selection and collection schedules are influenced by such factors as tidal currents and periodicity, and salinity distribution, rather than (river) currents, riffles, and pools.

2 Freshwater organisms are in general smaller, and the water is seldom chemically corrosive on equipment. Site selection in streams involves riffles, falls, pools, etc., and a unidirectional flow pattern. Lake collection may involve less predictable stratification or flow patterns.

E Definite objectives should be established in advance as to the size range of organisms to be collected and counted, i.e.: microscopic only, microscopic and macroscopic, those retained by "30 mesh" screens, invertebrates and/or vertebrates, etc.

II STANDARD PROCEDURES

A Certain standard supplementary procedures are a part of all field techniques. In order to be interpreted and used, every collection must be associated with a record of environmental conditions at the time of collection.

1 Data recorded should include the following as far as practicable.

Location (name of river, lake, etc.)

Station number (particular location of which a full description should be on record)

Date and hour

Air temperature

Water temperature (at various depths, if applicable)

Salinity (at various depths, if applicable)

Tidal flow (ebb or flood)

Turbidity (or light penetration, etc.)

Weather

Wind direction and velocity

Sky or cloud cover

Water color

Depth

Type of bottom

Type of collecting device and accessories

Method of collecting

Type of sample (quantitative or qualitative)

Number of samples at each station

Chemical and physical data, e.g., dissolved oxygen, nutrients, pH, etc.

Collector's name

Miscellaneous observations (often very important)

2 All collecting containers should be identified at least with location, station number, sample number, and date. Spares are very handy.

3 Much transcription of data can be eliminated by using sheets or cards with a uniform arrangement for including the above data. The same field data sheet may include field or laboratory analysis.

B Compact kits of field collecting equipment and materials greatly increase collecting efficiency, especially if collection site is remote from transportation.

III PERSONAL OBSERVATION AND PHOTOGRAPHY

A Direct or indirect observation of underwater conditions has become relatively efficient.

1 Diving spheres, pioneered by William Beebe, Cousteau, Honot, Willm, and Manad are proving very important for deep water observations.

2 Use of the aqualung permits direct personal study down to over 200 feet.

3 Underwater television (introduced by the British Admiralty for military purposes) is now generally available for biological and other observations.

4 Underwater photography is improving in quality and facility.

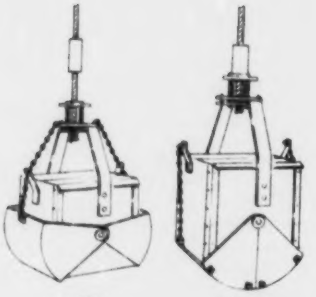
5 Underwater swimming or use of SCUBA is quite valuable for direct observation and collecting.

IV COLLECTION OF BOTTOM OR BENTHIC ORGANISMS

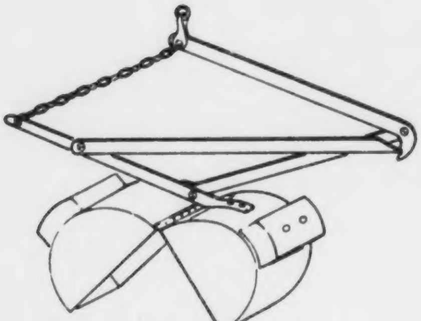
A Shoreline or Wading Depth Collecting Plates I, II

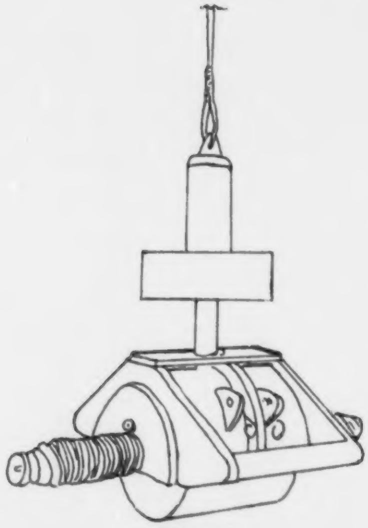
1 Hand picking of small forms attached to or crawling on rocks, sticks, etc. when lifted out of the

BOTTOM GRABS


open closed

Ekman


Petersen


Shipek

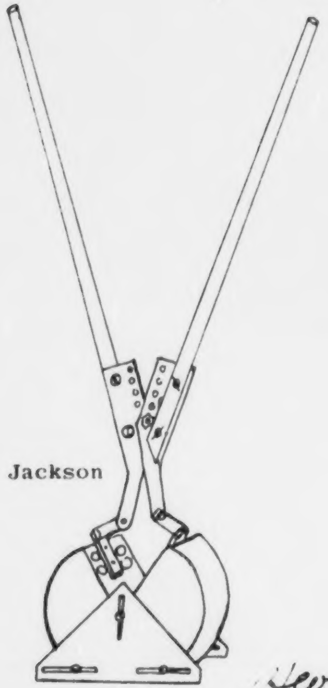

Jackson

PLATE I

LIMNOLOGICAL EQUIPMENT

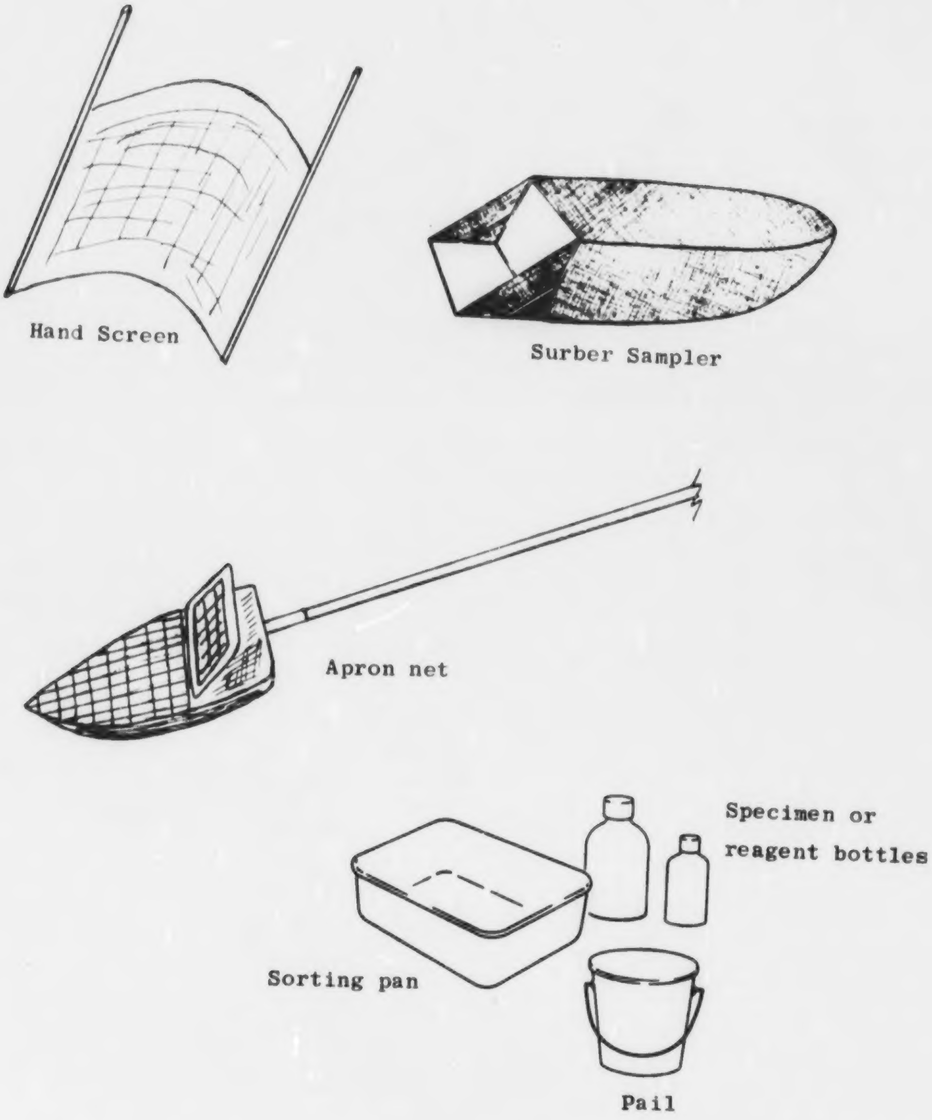

Hand Screen
Surber Sampler
Apron net
Specimen or
reagent bottles
Sorting pan
Pail

PLATE II

water is a fundamental and much used method for quickly assaying what is present and what may be expected on further search.

2 Patches of seaweed and eelgrass and shallow weedy margins anywhere are usually studied on a qualitative basis only.

  a The apron net is one of the best tools for animals in weed beds or other heavy vegetation. It is essentially a pointed wire sieve on a long handle with coarse screening over the top to keep out leaves and sticks.

  b Grapple hooks or a rake may be used to pull masses of vegetation out on the bank where the fauna may be examined and collected as they crawl out.

  c Quantitative estimates of both plants and animals can be made with a "stove pipe" sampler which is forced down through a weed mass in shallow water and embedded in the bottom. Entire contents can then be bailed out into a sieve and sorted.

  d A frame of known dimensions may be placed over an area to be sampled and the material within cropped out. This is especially good for larger plants and large bivalves. This method yields quantitative data.

3 Sand and mud flats in estuaries and shallow lakes may be sampled quantitatively by marking off a desired area and either digging away surrounding material or excavating the desired material to a measured depth. Handle-operated samplers recently developed by Jackson and Larrimore, make for more effective sampling of a variety of bottoms down to the depth of the handles. Such samples are then washed through graded screens to retrieve the organisms.

4 Ekman grabs are most useful on soft bottoms. This is a completely closing clamshell type grab with spring operated jaws. Size of grab is usually 6" × 6" or 9" × 9", the 12" × 12" size is impractical due to its heavy weight when filled with bottom material.

For use in shallow water, it is convenient to rig an Ekman with a handle and a hand operated jaw-release mechanism.

5 The Petersen type grab (described below) without weights will take satisfactory samples in firm muds, but tends to bury itself in very soft bottoms. It is seldom used in shallow water except as noted below.

B Collecting in Freshwater Riffles or Rapids

1 The riffle is one of the most satisfactory habitats for comparing stream conditions at different points.

2 The hand screen is the simplest and easiest device to use in this situation. Resulting collections are qualitative only.

  a In use the screen is firmly planted in the stream bed. Upstream bottom is thoroughly disturbed with the feet, or worked over by hand by another person. Organisms dislodged are carried down into the screen.

  b Screen is then lifted and dumped into sorting tray or collecting jar.

3 The well-known square foot Surber sampler is one of the best quantitative collecting devices for riffles.

a It consists of a frame one foot square with a conical net attached. It is usable only in moving water.

b In use it is firmly planted on the bottom. The bottom stones and gravel within the square frame are then carefully gone over by hand to ensure that all organisms have been dislodged and carried by the current into the net. A stiff vegetable brush is often useful in this regard.

c From three to five square-foot samples should be taken at each station to insure that a reasonable percentage of the species present will be represented.

4 The Petersen type grab may be used in deep swift riffles or where the Surber is unsuitable.

a It is planted by hand on the bottom, and worked down into the bottom with the feet.

b It is then closed and lifted by pulling on the rope in the usual manner.

5 A strong medium weight dipnet is the closest approach to a universal collecting tool.

a Sweeping Weed beds and Stream Margins

This is used with a sweeping motion, through weeds, over the bottoms or in open water. A triangular shape is preferred by some.

b Stop net or Kicking Technique

This may be used as a roughly quantitative device in riffles by holding the end flat against the bottom and backing slowly up-stream disturbing the substrate with one's feet. A standard period of time is used.

c The handle should be from 4 to 6 feet long, and about the weight of a garden rake handle.

d The ring should be made of steel or spring brass, and securely fastened to the handle. It should be strong but not cumbersome; size of ring stock will depend on diameter of ring.

e The bag or net should be the strongest available, not over 1/8 inch mesh, preferably about 1/16 inch. Avoid 30 or more meshes to the inch; this is so fine that the net plugs too easily and is slow and heavy to handle.

f There should be a wide canvas apron sewed around the rim and protecting the bag. The rim may be protected with leather if desired.

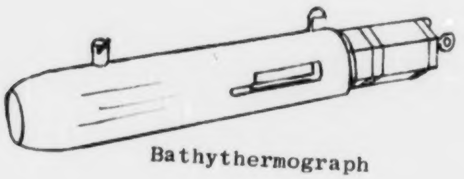
D Deep Water Benthic Collecting Plate III

1 When sampling from vessels, a crane and winch, either hand or power operated, is used. The general ideas described for shallow waters apply also to deeper waters, when practicable.

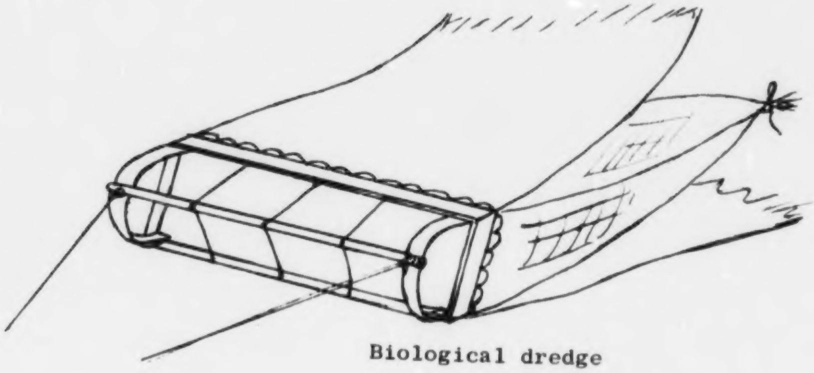
2 The Petersen type grab, seems to be the best all around sampler for the greatest variety of bottoms at all depths, from shoreline down to over 10, 000 meters. (Plate I)

a It consists of two heavily constructed half cylinders closed together by a strong lever action.

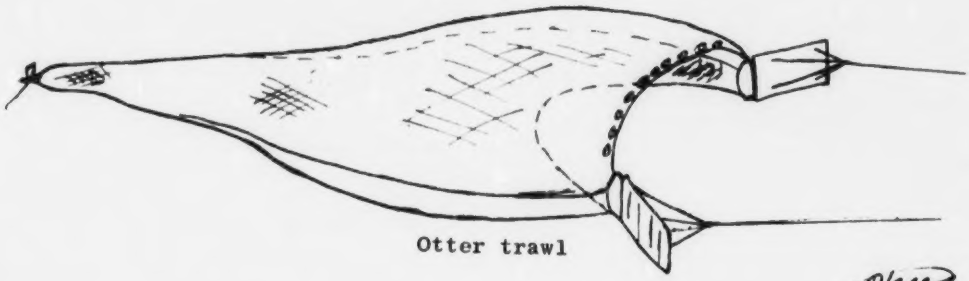
DEEP WATER EQUIPMENT



Bathythermograph



Biological dredge



Otter trawl

PLATE III

b To enable them to bite into hard bottoms, or to be used in strong currents, weights may be attached to bring the total weight up to between 50 and 100 lbs.

c Areas sampled range from 1/5th to 1/20th square meters (1/10 square meter equals approximately 1.1 square ft.)

d A Petersen grab to be hauled by hand should be fitted with 5/8 or 3/4 inch diameter twisted rope in order to provide adequate hand grip. It is best handled by means of wire ropes and a winch.

3 Other bottom samplers include the VanVeen, Lee, Holme, Smith-McIntyre, Knudsen, Ponar, and others.

4 A spring loaded sampler has recently been developed by Shipek for use on all types of bottoms. It takes a half-cylinder sample, 1/25th square meters in area and approximately 4 inches deep at the center. The device is automatically triggered on contact with the bottom, and the sample is completely protected enroute to the surface. (Plate I)

5 Drag dredges or scrapes are often used in marine waters and deeper lakes and streams, and comprise the basic equipment of several types of commercial fisheries. Some types have been developed for shallow streams. In general however, they have been little used in fresh water.

6 The above is only a partial listing of the many sampling devices available. Others that are often encountered are the orange-peel bucket, plow dredge, scallop type dredge, hydraulic dredges, and various coring devices. Each has its own advantages and disadvantages and it is up to the worker and his operation to decide what is best for his particular needs. The Petersen type and Ekman grabs are perhaps the most commonly used.

7 Traps of many types are used for various benthic organisms, especially crabs and lobsters. Artificial substrates (below) are in essence a type of trap.

8 Since most biological communities are not evenly distributed, it is advisable to routinely take at least two and preferably more samples from any one station.

E Artificial substrates rely on the ecological predilection of organisms to grow wherever they find a suitable habitat. When a small portion of artificial habitat is provided, it tends to become populated by all available species partial to that type of situation. The collector can then at will remove the habitat or trap to his laboratory and study the population at leisure.

This versatile research technique is much used for both routine monitoring and exploratory studies of pollution. It is also exploited commercially, especially for shellfish production. Types of materials used include:

1 Cement plates and panels.

2 Wood (especially for burrowing forms).

3 Glass slides (ex: Catherwood diatometer).

4 Multiple plate trap (masonite).

5 Baskets (or other containers) holding natural bottom material and either imbedded in the bottom, or suspended in the overlying water.

6 Unadorned ropes suspended in the water, or sticks thrust into the bottom.

F Sorting and Preservation of Collections

1 Benthic collections usually consist of a great mass of mud and other debris among which the organisms are hidden. Various procedures may be followed to separate the organisms.

a The organisms may be picked out on the spot by hand or the entire mess taken into the laboratory where it can be examined more efficiently (especially in rough weather). Roughly equivalent time will probably be required in either case.

b Specimens may be simply observed and recorded or they may be preserved as a permanent record.

c Organisms may be simply counted, weighed, or measured volumetrically; or they may be separated and recorded in groups or species.

2 If separation is in the field, this is usually done by hand picking, screening, or some type of flotation process.

a Hand picking is best done on a white enameled tray using light touch limnological forceps.

b Screening is one of the most practical methods to separate organisms from debris in the field. Some prefer to use a single fine screen, others prefer a series of 2 or 3 screens of graded sizes. The collection may be dumped directly on the screen and the mud and debris washed through, or it may be dumped into a bucket or small tub. Water is then added, the mixture is well stirred, and the supernatant poured through the screen. The residue is then examined for heavy forms that will not float up.

c A variation of this method in situations where there is no mud is to pour a strong sugar or salt solution over the collection in the bucket, stir it well, and again pour the supernatant through the screen. This time, however, saving the flotation solution for re-use. The heavier-than-water solution accentuates the separation of organisms from the debris (except for the heavy shelled molluscs, etc.). A solution of 2-1/2 lbs. of sugar per gallon of water is considered to be optimum.

3 Preservation or stabilization is usually necessary in the field.

a 95% ethanol (ethyl alcohol) is highly satisfactory. A final strength of 70% is necessary for prolonged storage. If the collection is drained of water and flooded with 95% ethanol in the field, a laboratory flotation separation can usually be made later, thus saving much time. Considerable quantities of ethanol are required for this procedure.

b Formaldehyde is more widely available and is effective in concentrations of 3 - 10% of the commercial formulation. However, it shrinks and hardens specimens, collector, and laboratory analyst without favor! In order to minimize bad effects from formalin, neutralized formalin is recommended. Mollusc shells will eventually disintegrate in acid formalin

c Properly preserved benthos samples may be retained indefinitely, thereby enhancing their utility.

d Refrigeration or icing is very helpful.

V MICROFAUNA AND PERIPHYTON (OR AUFWUCHS) SAMPLING

A This is a relatively new area which promises to be of great importance. The microfauna of mud and sand bottoms may be studied to some extent from collections made with the various devices mentioned above. In most cases however, there is considerable loss of the smaller forms.

B Most special microfauna samplers for soft bottoms are essentially modified core samplers in which an effort is made to bring up an undisturbed portion of the bottom along with the immediately overlying water. The best type currently seems to be the Enequist sampler which weighs some 35 kg. and takes a 100 sq. cm sample 50 cm. deep.

C Microfauna from the surface of hard sand or gravel bottoms may be sampled by the Hunt vacuum sampler. This has a bell-shaped "sampling" tube sealed by glass diaphragm. On contact with the bottom, the glass is automatically broken and the nearly bottom material is swept up into a trap.

D Periphyton attached to or associated with hard surfaces such as rock or wood may be sampled by scraping or otherwise removing all surface material from a measured area. The periphyton, however, is more effectively quantitatively sampled by artificial substrate techniques described above.

VI THE COLLECTION, OR SAMPLING OF PLANKTON PLATE IV

A Phytoplankton: A Planned Program is Desirable

1 A planned program of plankton analysis should involve periodic sampling at weekly or even more frequent intervals.

2 A well-planned study or analysis of the growth pattern of plankton in one year will provide a basis for predicting conditions the following year since seasonal growth patterns tend to repeat themselves from year to year.

a Since the seasons and the years differ, records accumulated over the years become more useful.

b As the time for an anticipated bloom of some troublesome species approaches, the frequency of analyses may be increased.

3 Detection of a bloom in its early stages will facilitate more economical control.

B Field Aspects of the Analysis Program

1 Two general aspects of plankton analysis are commonly recognized: quantitative and qualitative.

a Qualitative examination tells what is present.

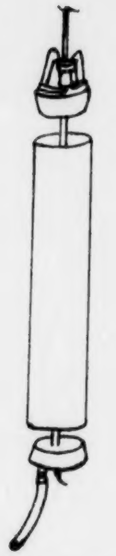
b Quantitative tells how much.

c Either approach is useful, a combination is best.

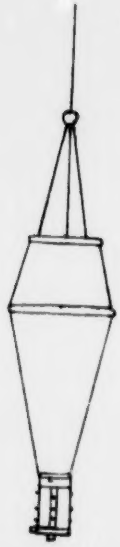
2 Equipment for collecting samples in the field is varied.

a A half-liter bottle will serve for surface samples of phytoplankton, if carefully taken.

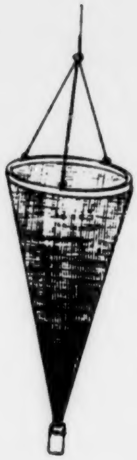
PLANKTON SAMPLERS

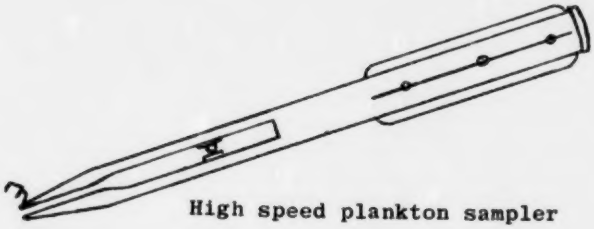


Kemmerer



Wisconsin net





High speed plankton sampler

PLATE IV

b A Kemmerer, Nansen, or other special sampler (small battery operated pumps are time saving) is suggested for depth samples.

c Plankton nets concentrate the sample in the act of collecting and also capture certain larger forms which escape from the bottles. Only the more elaborate types are quantitative however. For phytoplankton, #20 or #25 size nets are commonly used. Usually a net diameter of 5 - 10 inches is sufficient. The smaller forms however, are lost through any net.

C Zooplankton Collecting

1 Since zooplankton have the ability to swim away from water bottles, etc. nets towed at moderately fast speed are used for their capture. Number 12 nets (aperature size 0.119 mm, 125 meshes 1 inch) or smaller numbered net sizes are commonly used. A net diameter greater than 5" is preferred.

Frequently half meter nets or larger are employed. These may be equipped with flow measuring devices for measuring the amount of water entering the net.

2 Other instruments such as the Clark-Bumpus, Gulf-Stream, Hardy continuous plankton recorder, and high-speed instruments are used for collecting zooplankton, also.

3 The devices used for collecting plankton capture both the plant and animal types. The mesh size (net no.) is a method for selecting which category of plankton is to be collected.

D The Location of Sampling Points

1 Both shallow and deep samples are suggested.

a "Shallow" samples should be taken at a depth of 6 inches to one foot. The surface film is often significant.

b "Deep" samples should be taken at such intervals between surface and bottom as circumstances dictate. In general, the entire water column should be sampled as completely as practicable, and the plankton from each level recorded separately.

2 For estuarine plankton, it is necessary to sample different periods in the stage of the tide, otherwise samples would be biased to a given time, or type of water carried by the tidal currents.

3 Plankton is subjected to the force of the winds and currents. As a result, the plankton is often in patches or "wind rows" (Langmuir cells). For this reason when using a net, it is often desirable to tow the net at right angles to the wind or current.

4. Nearly all plankton are horizontally discontinuous. Planktonic organisms tend to be numerous near the bottom in daylight, but distributed more evenly through the water column at night. Therefore, a series of tows or samples at different depths is necessary to obtain a complete sampling. One technique often employed is to take an oblique tow from the bottom to the top of the water column.

5. Pilot studies to indicate sampling locations and intervals are often mandatory. Some studies require random sampling points.

6. The number of sampling stations that should be estabilshed is limited by the capability of the laboratory to analyze the samples, but should approach the needs of the objectives as closely as possible.

7 Field conditions greatly affect the plankton, and a record thereof should be carefully identified with the collection as in II above.

8 Provisions should be made for the field stabilization of the sample until the laboratory examination can be made if more than an hour or so is to elapse.

   a Refrigeration or icing is very helpful, but ice should never be placed in the sample.

   b Preservation by 5% formalin is widely used but badly shrinks animals and makes all forms brittle.

   c Lugols solution is a good preservative.

   d Ultra-violet sterilization is sometimes used in the laboratory to retard the decomposition of plankton.

   e A highly satisfactory merthiolate preservative has been described by Weber (1968).

VII COLLECTING FISH AND OTHER NEKTON PLATES V, VI

A Fish and other nekton must be sought in the obscure and unlikely areas as well as the obvious locations in order for the collection to be complete. Several techniques should be employed whereever possible (this is appropriate for all biota). It is advisable to check with local authorities to inform them of the reasons for sampling, because many of the techniques are not legal for the layman. In this area, perhaps more than any other, professionally trained workers are important. Also, there must be at least one helper, as a single individual always has difficulty in pulling both ends of a 20 foot seine simultaneously! The more common techniques are listed below.

B Seines

1 Straight seines range from 4-6 feet and upwards in length. "Common sense" minnow seines with approximately 1/4 inch mesh are widely used along shore for collecting the smaller fishes.

2 Bag seines have an extra trap or bag tied in the middle which helps trap and hold fish when seining in difficult situations.

C Gill nets are of use in offshore and/or deep waters. They range in length from approximately 30 yards upward. A mesh size is designed to catch a specified size of fish. The trammel net is a variation of the gill net.

D Traps range from small wire boxes or cylinders with inverted cone entrances to semi-permanent weirs a half mile or more in length. All tend to induce fish to swim into an inner chamber protected by an inverted cone or V - shaped notch to prevent escape. Current operated rotating fish traps are also very effective (and equally illegal) in suitable situations.

E Trawls are submarine nets, usually of considerable size, towed by vessels at speeds sufficient to overtake and scoop in fish, etc. The mouth of the net must be held open by some device such as a long beam (beam trawl) or two or more vanes or "otter boards" (otter trawl). Plate III

1 Beam and otter trawls are usually fished on the bottom, but otter trawls when suitably rigged are now being used to fish mid-depths.

2 The midwater trawl resembles a huge plankton net many feet in diameter. It is proving very effective for collecting at mid-depths.

FISH NETS

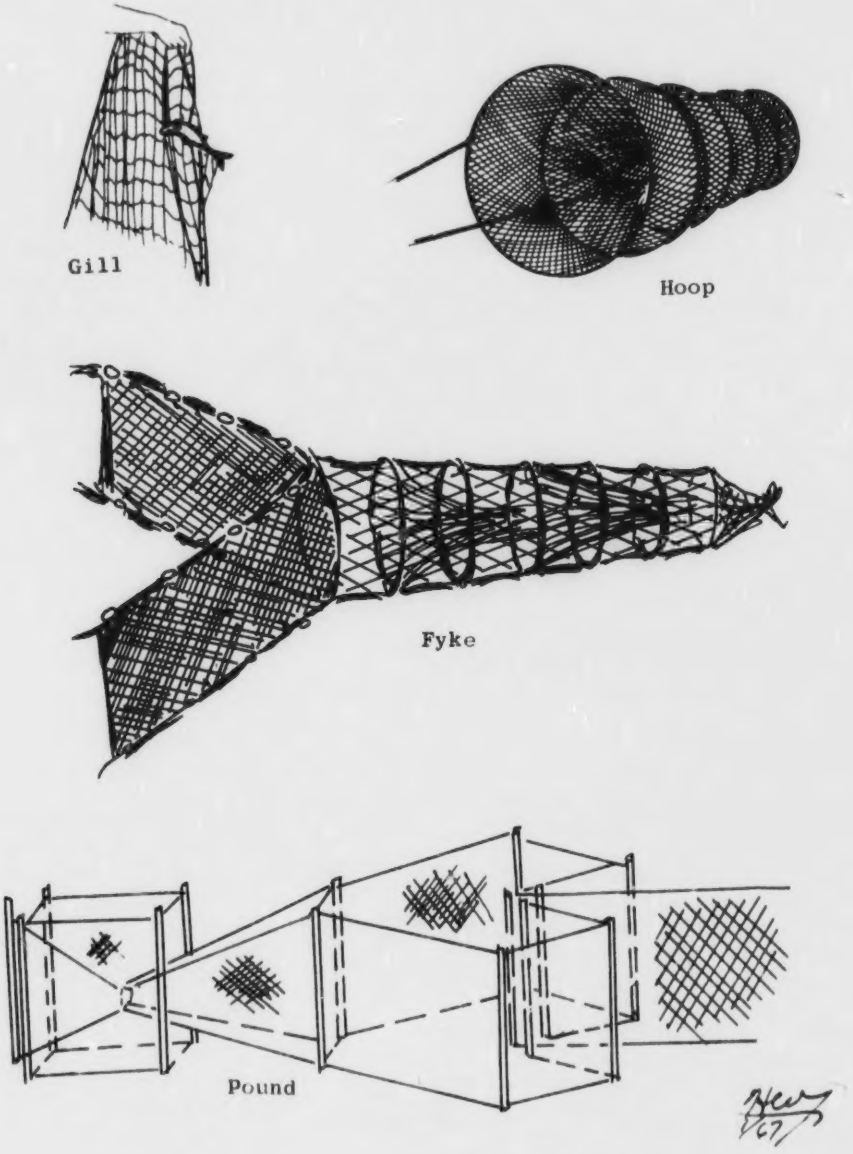

Gill
Hoop
Fyke
Pound

PLATE V

MIDWATER TRAWL

PLATE VI

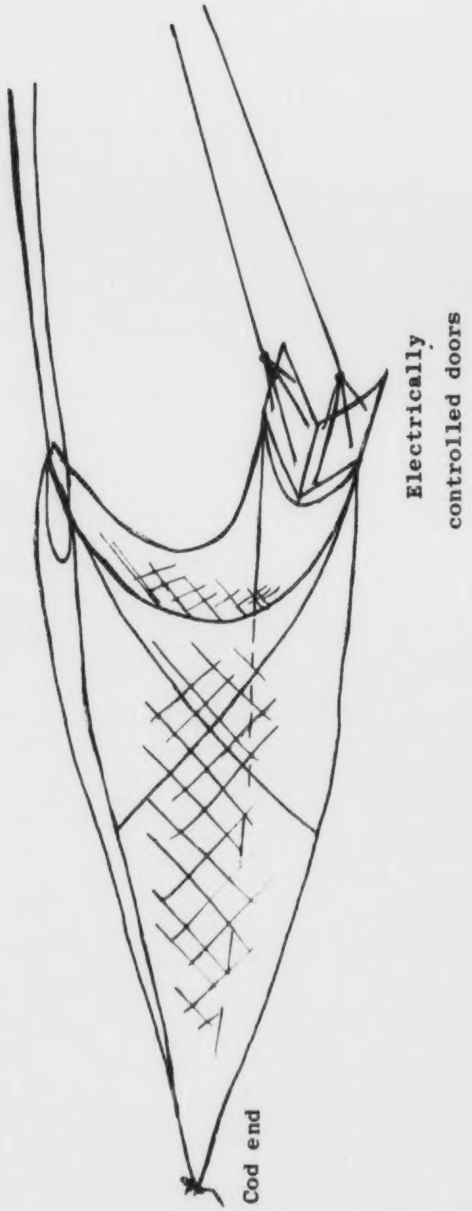

Electrically controlled doors
Cod end

PLATE VI

Numerous special designs have been developed. Plate VI

F Electric seines and screens are widely employed by fishery workers in small and difficult streams. They may also be used in shallow water like areas with certain reservations.

G Poisoning is much used in fishery studies and management. Most widely used and generally satisfactory is rotenone in varying formulations, although many others have been employed from time to time, and some appear to be very good. Under suitable circumstances, fish may even be killed selectively according to species.

H Personal observation by competent personnel, and also informal inquiries and discussions with local residents will often yield information of real use. Many laymen are keen observers, although they do not always understand what they are seeing. The organized creel census technique yields data on what and how many fish are being caught.

I Angling remains in its own right a very good technique in the hands of the skilled practitioner, for determining what fish are present. Spear-fishing also is now being used in some studies.

J Fish and other nekton are often tagged to trace their movements during migration and at other times. Miniature radio transmitters can now be attached or fed to fish (and other organisms) which enable them to be tracked over considerable distances. Physiological information is often obtained in this way. This is known as telemetry.

VIII SPECIAL REQUIREMENTS ON BOATS

Handling biological collections (as contrasted to chemical and physical sampling) on board boats differs with the size of the craft and the magnitude of operations. Some possible items are listed below. Hoisting and many other types of gear are used in common with other types of collection, and will not be listed.

A Special Laboratory Room(s)

B Constant flow of Clean water for culturing organisms. (Selection of materials and design of a system to insure non-toxic water may be very troublesome but very important.)

C Live Box built into ship at water level

D Refrigeration System(s)

  1 For controlling temperature of experimental organisms in laboratory.

  2 For deep-freezing and storage of specimens to be examined later.

E Storage Space (Unrefrigerated)

F Facilities for the safe storage and use of microscopes and other laboratory equipment.

G Facilities for the safe storage and use of deck equipment.

H Administrative access to the Captain and Technical Leader in order to coordinate requirements for biological collection (such as a slow plankton tow) with those for other collections.

I Safety of personnel working in and around boats, as well as in other field activities should be seriously considered and promoted at all times.

IX OTHER TYPES OF BIOLOGICAL FIELD STUDIES INCLUDE

A Productivity Studies of Many Types

B Life Cycle and Management

C Distribution of Sport or (potentially) Commercial Species

D Scattering Layers and Other Submarine Sound Studies

E Artificial Culture of Marine Food Crops

F Radioactive Uptake

G Growth of Surface-Fouling Organisms

H Marine Borers

I Dangerous Marine Organisms

J Red Tides

K Others

X SOURCES OF COLLECTING EQUIPMENT

Many specialized items of biological collecting equipment are not available from the usual laboratory supply houses. Consequently, the American Society of Limnology and Oceanography has compiled a list of companies handling such items and released it as "Special Publication No. 1, Sources of Limnoligical and Oceanographic Apparatus and Supplies." Available from the Secretary of the Society.

XI SAFETY

The hazards associated with work on or near water require special consideration. Personnel should not be assigned to duty alone in boats, and should be competent in the use of boating equipment (courses are offered by the U. S. Coast Guard). Field training should also include instructions on the proper rigging and handling of biological sampling gear.

Life preservers(jacket type work vests) should be wron at all times when on or near deep water. Boats should have air-tight or foam-filled compartments for flotation and be equipped with fire extinguishers, running lights, oars, and anchor. The use of inflatable plastic or rubber boats is discouraged.

All boat trailers should have two rear running and stop lights and turn signals and a license plate illuminator. Trailers 80 inches (wheel to wheel) or more wide should be equipped with amber marker lights on the front and rear of the frame on both sides.

Laboratories should be provided with fire extinguishers, fume hoods, and eye fountains. Safety glasses should be worn when mixing dangerous chemicals and preservatives.

A copy of the EPA Safety Manual is available from the Office of Administration, Washington, D.C. (Reference: 10)

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Descriptors:
Aquatic Environment, Analytical Techniques, Sampling

SPECIAL APPLICATIONS AND PROCEDURES FOR BIOASSAY

I INTRODUCTION

A The report of the Council on Environmental Quality (1970) repeatedly stresses the need for the development of predictive, simulative, and managerial capabilities to combat air and water pollution. The last capability depends on the first two.

B The standard static jar fish bioassay, which uses death as a response, enables one to predict the toxicity of a particular waste to fish. One limitation of this procedure is that it uses a grab sample which represents the quality of the waste at only one point in time. The water used to make the dilutions is also taken at one point in time. At the actual industrial site, the quality of the waste and the river water vary through time. A composite waste sample partially overcomes this limitation, but may mask variations that are biologically important.

C One could put fish in a continuous flow of waste diluted with river water, but then there is one further limitation of the standard bioassay: death is used as the response. In order to prevent damage to organisms, it is necessary to have an early warning of dangerous conditions, so that corrective action can be taken. In other words, symptoms of ill health, which occur before death, must be detected if there is to be time for diagnosis and treatment.

II METHODS AND MATERIALS

A Fish Movement Patterns

1 Fish movement patterns can be monitored using the technique of light beam interruption described in detail by Cairns, et al. (1970). Dawn and dusk are simulated by a motor-driven dimming unit which gradually increases the intensity of the room lights over a half-hour period starting at 6:30 a.m. and gradually decreases the intensity to 0 over a half-hour period starting at 6:30 p.m. The cumulative movement of each of six bluegill sunfish, a single fish per tank, is recorded every hour throughout a test except during the simulated sunrise and sunset when an additional record is made on the half hour. Each day is divided into four intervals; first half day, second half day, first half night and second half night (Table I). Before any statistical analysis can be performed, recordings for day 1 must be completed. After the cumulative movement for day 1 is recorded, statistical analyses are performed after the completion of each designated time interval. For example, the cumulative movement recorded hourly for each fish during day 1, first half day values are compared to the cumulative movement recorded hourly for each fish during day 2, first half day values.

2 Based on the results of 20 laboratory experiments "stress detection" is defined as the presence of two or more abnormal movement patterns recorded during the same time interval.

B Fish Breathing

1 Breathing rates may be determined from polygraph recordings of breathing signals. The fish are tested in plexiglas tubes through which dechlorinated tap water or some toxic solution is metered at a flow rate of approximately 100 ml/min. Breathing signals are detected by three platinum wire electrodes placed in the water; an active electrode, an indifferent electrode, and a ground. The test chambers and methods of acclimating the fish are described in more detail by Cairns, et al. (1970) The photoperiod is the same as that for the fish movement study.

2 The fish are placed in test chambers by 6:00 p.m. and the recordings began at 6:00 a.m. the next day to allow the fish to recover overnight from handling. Toxic solutions are introduced at 10:00 a.m. after the experimental fish have been exposed to water containing no added

toxicant for periods of one to six days. Each experimental fish thus serves as its own control. In addition, one or two fish are never exposed to the toxicant and serve as controls throughout each experiment. In one experiment, using zinc as the toxicant, reported in Table VI, six control fish were exposed to water containing no added zinc for four days.

a Preliminary evidence suggested that the data could be analyzed by separating the experimental day into four periods; a period from 6:00 to 8:00 a.m. when the breathing rates changed markedly, a period from 9:00 a.m. to 5:00 p.m. when the rates were comparatively high, another period of rapid change from 6:00 to 8:00 p.m., and a night period from 9:00 p.m. to 5:00 a.m. when the rates were comparatively low (Sparks, et al., 1970).

b Bluegills increase their breathing rates when exposed to zinc (Cairns, et al., 1970). An individual fish was thus considered to have shown a response each time its breathing rate during a time period exceeded the maximum breathing rate observed during the corresponding period of the first day, before any zinc was added. A response was scored for each value on the second day that was higher than the first day maximum for the comparable period. The control periods (before any zinc was added) and the experiment where no zinc was added at all were used to determine how many false detections this method of analysis would produce. The experimental periods (after zinc was added) determined how quickly the method of analysis could detect zinc concentrations in water.

c Zinc concentrations were determined daily by atomic absorption spectrophotometry.

III RESULTS

A Fish Movement Patterns

1 Table 1 shows the results of one continuous flow experiment carried out for 20 days. During this experiment fish were exposed to zinc on day 7 from 1:00 p.m. until 7:00 p.m. at which time the flow was returned to normal dilution water. The zinc concentrations reached their maximum at 7:00 p.m. and atomic absorption analyses on effluent samples collected at this time showed the following concentrations: tank one, 13.32; tank two, less than 0.03; tank three, 11.39; tank four, 12.72; tank five, 13.32; and tank six, 12.59 mg/l Zn^{++}. The results show that these concentrations of zinc developing over the six hour interval of exposure were insufficient to cause a detectable change in the movement patterns of the fish. By 8:30 a.m. of day 8 the effluent zinc concentrations were less than 0.30 in all cases.

2 To determine the percent survival and recovery patterns of the fish once stress detection occurred, zinc flow was reinitiated at 1:00 p.m. on day 13 of this experiment. Between 8:00 and 9:00 p.m. on day 13 the zinc concentration in the effluent reached a maximum of: 7.51 for tank one; less than 0.05 for tank two; 7.49 for tank three; 7.52 for tank four; 7.49 for tank five; and 7.54 mg/l for tank six. The concentrations remained near the above values until the statistical analyses showed "stress detection" during the first half night values on day 14 (Table 1). As soon as stress detection occurred the flow was returned to normal dilution water. At 10:00 a.m. on day 15 zinc analyses showed all effluent concentrations to be less than 0.70 mg/l Zn^{++}. Stress detection continued to be registered for two consecutive time intervals following the initial detection, but after that no stress detection was registered and the frequency of abnormal patterns returned to prestress levels within 48 hours. In this experiment

as with all others in which dilution water containing zinc was replaced with dilution water minus zinc at that time of stress detection all fish survived!

3 The results from the series of experiments at progressively lower zinc concentrations indicate that the lowest detectable concentration is between 3.65 (Table II) and 2.93 mg/l zinc (Table III) for a 96-hour exposure.

B Fish Breathing

1 Table IV shows the breathing rates of five fish on days 1, 2, and 7 of experiment 8. The first four fish were exposed to a measured zinc concentration of 4.16 mg/l, beginning at 10 a.m. on day 7. The fifth fish served as a control and was not exposed to any added zinc. The amplitude of the breathing signals decreased every night, and the breathing rates for fish 2, in particular, could not be determined during some portions of the dark period (7:30 p.m. - 7:00 a.m.). The maximum breathing rates for each fish during each period of the first day are circled. The breathing rate of any fish during a time period of day 2 or day 7, which is greater than the maximum breathing rate recorded for that fish during the corresponding time period of the first day has a rectangle drawn around it. The total number of fish showing increased breathing is given at the bottom of each column. On day 2, fish 2 showed increased breathing on just two occasions. In contrast after zinc was added on day 7, three and four experimental fish at a time showed increased breathing.

2 Table V summarizes the results of successive comparisons of the first day maximal breathing rates to breathing rates on subsequent days (SCM method of analysis), for experiment 8. During the control period before any zinc was added there were 15 occasions when a single experimental fish responded, and three occasions when two experimental fish responded at the same time. At no time during the control period did more than two fish show responses together. After the zinc was introduced, all four of the exposed fish showed responses simultaneously on five occasions, and three fish showed responses during the same time interval on 19 occasions. If the criterion for detection of water conditions potentially harmful to fish were two or more responses during the same time period, then three false detections would have occurred before any zinc was added, and 4.16 mg/l zinc would have been correctly detected eight hours after it was introduced. If the detection criterion were three or more responses during the same time period, then no false detections would have occurred and the zinc would still have been correctly detected after eight hours.

3 The lowest zinc concentration tested was 2.55 mg/l. Using a detection criterion of simultaneous responses by three fish, this concentration was detected 52 hours after the zinc was added, with no false detections occurring during the four hours before zinc was added (Table VI). The responses of six control fish that were exposed to dilution water containing no added zinc are also shown for comparison. Note that there was no tendency toward increased breathing rates through time in the control fish, and that no more than one control fish showed an increased breathing rate during one time period.

4 Table VII summarizes information on three experiments that indicates the effectiveness of the SCM method of analysis when different criteria for detection are used. Changing the criterion for detection from one to three responses per time period generally increases the lag time and decreases the number of false detections. The lag time is the time from the addition of zinc to the first detection. A false detection is one occurring before any zinc is added to the water.

IV DISCUSSION

A The experiments described above show that the movements and breathing rates of bluegill

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sunfish can be used to detect sublethal concentrations of zinc. The criterion for detection is a certain number of fish showing an arbitrarily defined response in breathing rate or activity during one time period.

B In choosing a specific criterion for detection, the risk of not detecting stressful conditions soon enough must be weighed against the risk of false detections, and the choice would probably be determined by the nature of the pollutant. If a pollutant is easily detected by the biological monitoring system, is slow-acting, and if the toxic effects are reversible, then the criterion for detection might be responses by 3/4 of the test fish, to avoid the false detections that would necessitate expensive remedial action or a temporary shut-down. On the other hand, an industry that produces an effluent containing a fast-acting toxicant whose effects are irreversible would probably use a criterion that leads to rapid detection (responses by 1/4 to 1/2 of the test fish), and would have to go to the expense of installing holding ponds or recycling facilities to accommodate a relatively high number of false detections. Alternatively, a safety factor could be introduced by metering proportionally more waste into the dilution water delivered to the test fish than is delivered to the stream. The safety factor could be determined by growth and reproduction experiments with fish.

C In an actual industrial situation water and waste qualities are apt to vary unpredictably, and it would certainly be desirable to have a redundant detection system. It is conceivable that some harmful combination of environmental conditions and waste quality would be detected by monitoring one biological function, but not by monitoring another. It is also possible that excessive turbidity would disrupt the light beams of the movement monitor, and not affect the breathing monitor; or that an excessive concentration of electrolytes would affect the electrodes of the breathing monitor, but not affect the activity monitor. Therefore, the activity monitor and the breathing monitor have been combined in our laboratory for further experiments (Fig. 1).

D The rate of data acquisition and analysis could be greatly speeded up if the monitoring system were automated as shown in Figure 2. The sampling rate would be controlled by a minicomputer which could receive data from the movement monitor and the polygraph via a multiplexer as often as every minute. The minicomputer would be programmed to perform statistical analyses every 10 minutes, for example, and output the results on a teleprinter.

E Figure 3 shows how the fish monitoring units would be used at an actual industrial site. A monitoring unit would be located on each waste stream in the plant and on the combined waste stream. The experimental fish in each unit would be exposed to waste diluted with water from the river above the plant, and control fish would be exposed to upstream water alone (Fig. 4). The information from each monitoring unit could be analyzed by a central data processor, and when there was a warning response, the industry could tell which waste stream was at fault. If the problem was outside the plant, the control fish would show responses.

F Figure 5 shows how the in-plant monitoring systems would be integrated into a river management system. The in-plant monitoring units are shown as squares, and in addition to supplying information to each industry, the monitoring units also inform the control center. In such a system, there are several alternative damage prevention measures that could be used, in addition to whatever measures, such as shunting wastes to a holding pond or recycling wastes for further treatment, are available to each industry. If the monitoring units at Industry 2 indicate that toxic waste conditions are developing, then the control center might have Industry 1 hold its waste until the danger of combining wastes from Industry 1 and 2 in the river were alleviated by control measures at Industry 2. Alternatively, the control center might call for a release of water from the upstream dam to dilute the effluent from industry.

G It is likely that "fish sensors" in continuous monitoring units at industrial sites can warn of developing toxic conditions in time to forestall acute damage to the fish populations in streams. In conjunction with stream water quality standards for chronic exposure, such biological monitoring systems should make it possible for healthy fish populations to co-exist with industrial water use.

ACKNOWLEDGEMENT

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11 Cairns, John Jr.; Sparks, R. E.; and Waller, W. T. "A tentative proposal for a rapid in-plant biological monitoring system." in Biological Methods for the Assessment of Water Quality. ASTMSTP 526, American Society for Test. and Mat., 1973, pp. 127-147.

This outline was prepared by John Cairns, Jr. and Richard E. Sparks, Center for Environmental Studies and Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Descriptors:
Environmental Control, Bioassay, Toxicity, Fish Behavior, Fish, Monitoring

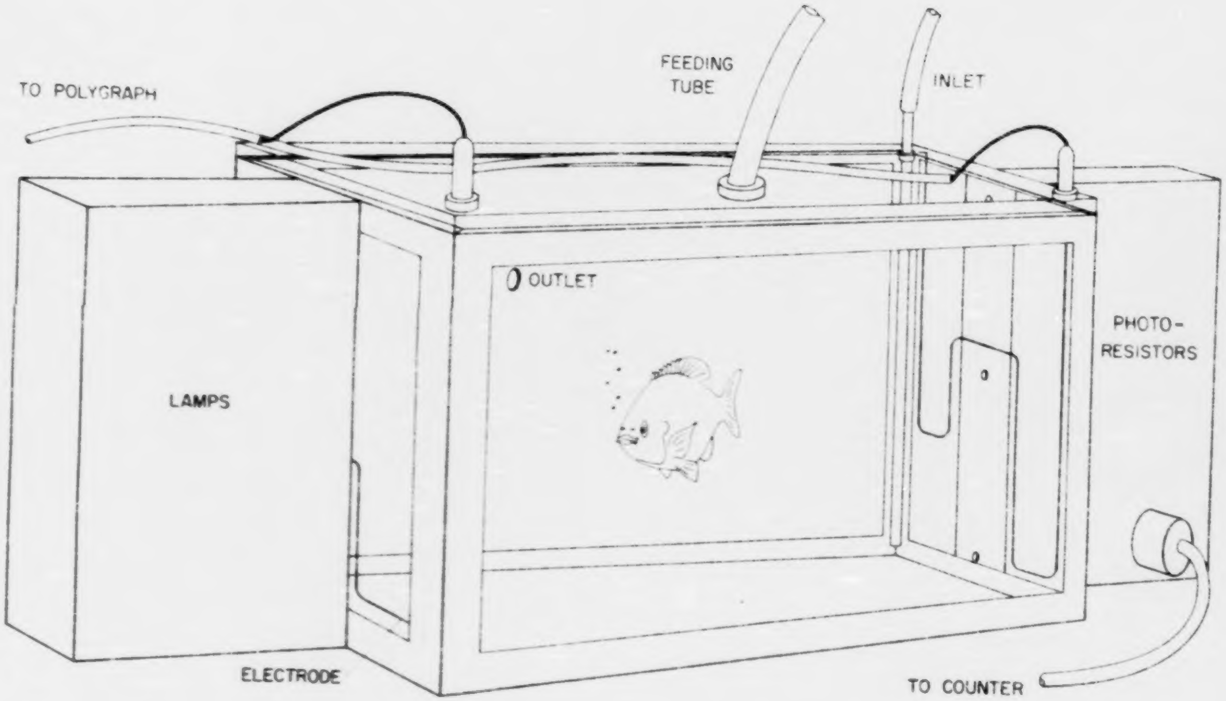

TO POLYGRAPH
FEEDING TUBE
INLET
OUTLET
PHOTO-RESISTORS
LAMPS
ELECTRODE
TO COUNTER

Figure 1. Test chamber for monitoring system, showing the electrodes for recording fish breathing and the light-beam system for recording fish movement.

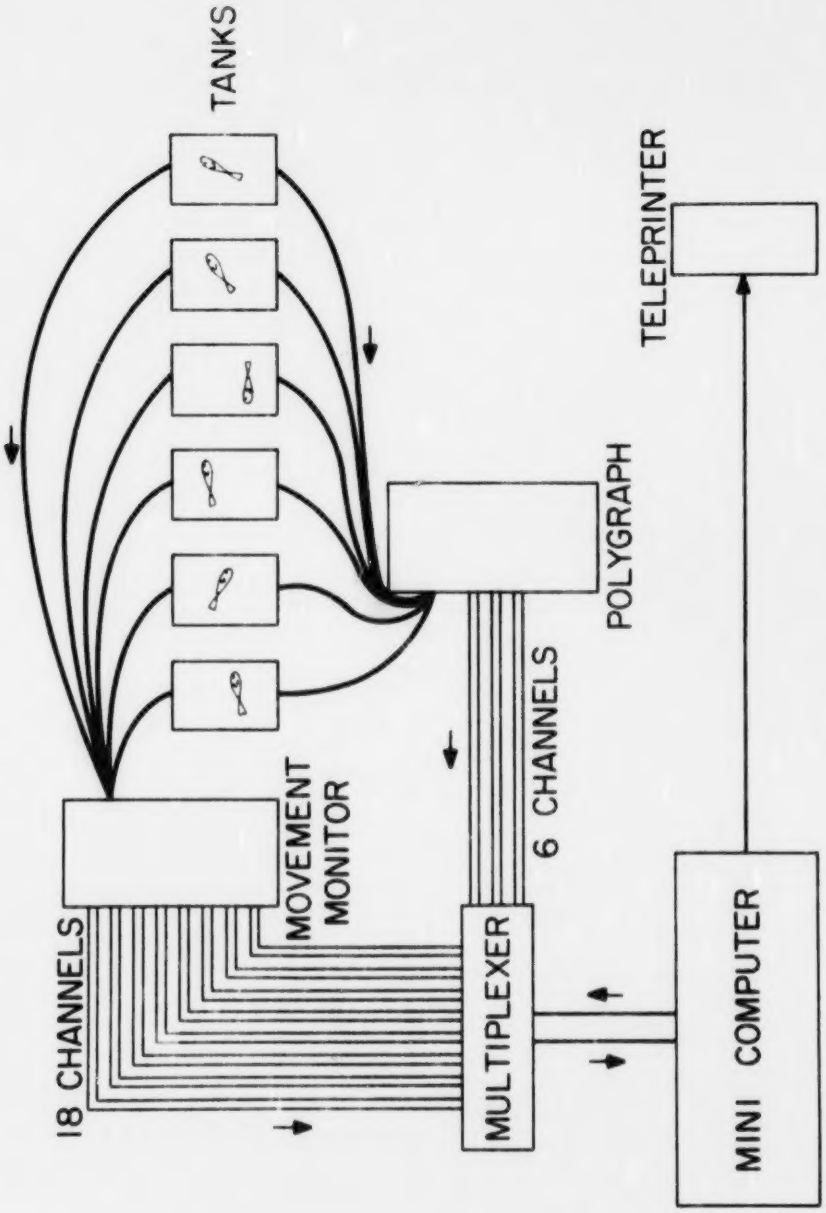

TANKS
TELEPRINTER
POLYGRAPH
6 CHANNELS
MOVEMENT MONITOR
18 CHANNELS
MULTIPLEXER
MINI COMPUTER

Figure 2. An automated fish monitoring unit.

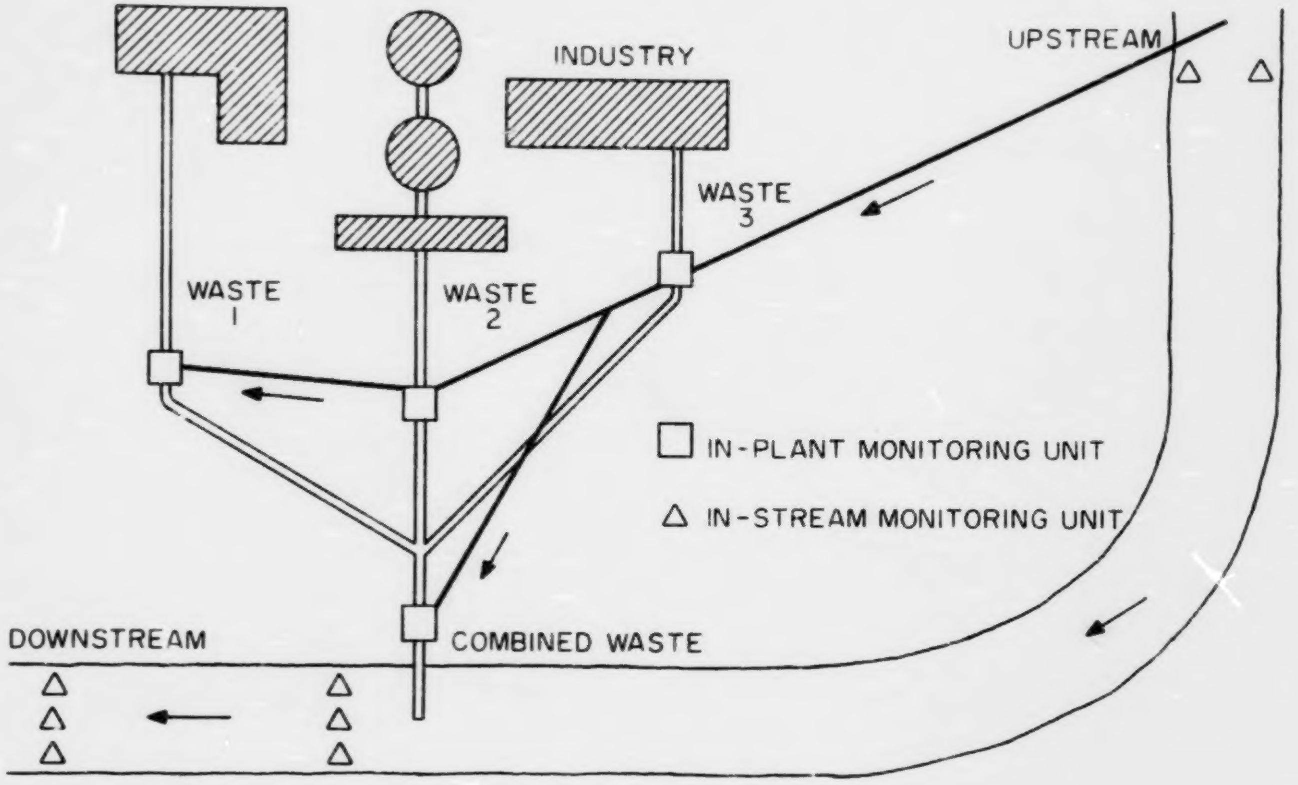

INDUSTRY
UPSTREAM
WASTE 3
WASTE 2
WASTE 1
□ IN-PLANT MONITORING UNIT
△ IN-STREAM MONITORING UNIT
DOWNSTREAM
COMBINED WASTE

Figure 3. Arrangement of fish monitoring units at an industrial site.

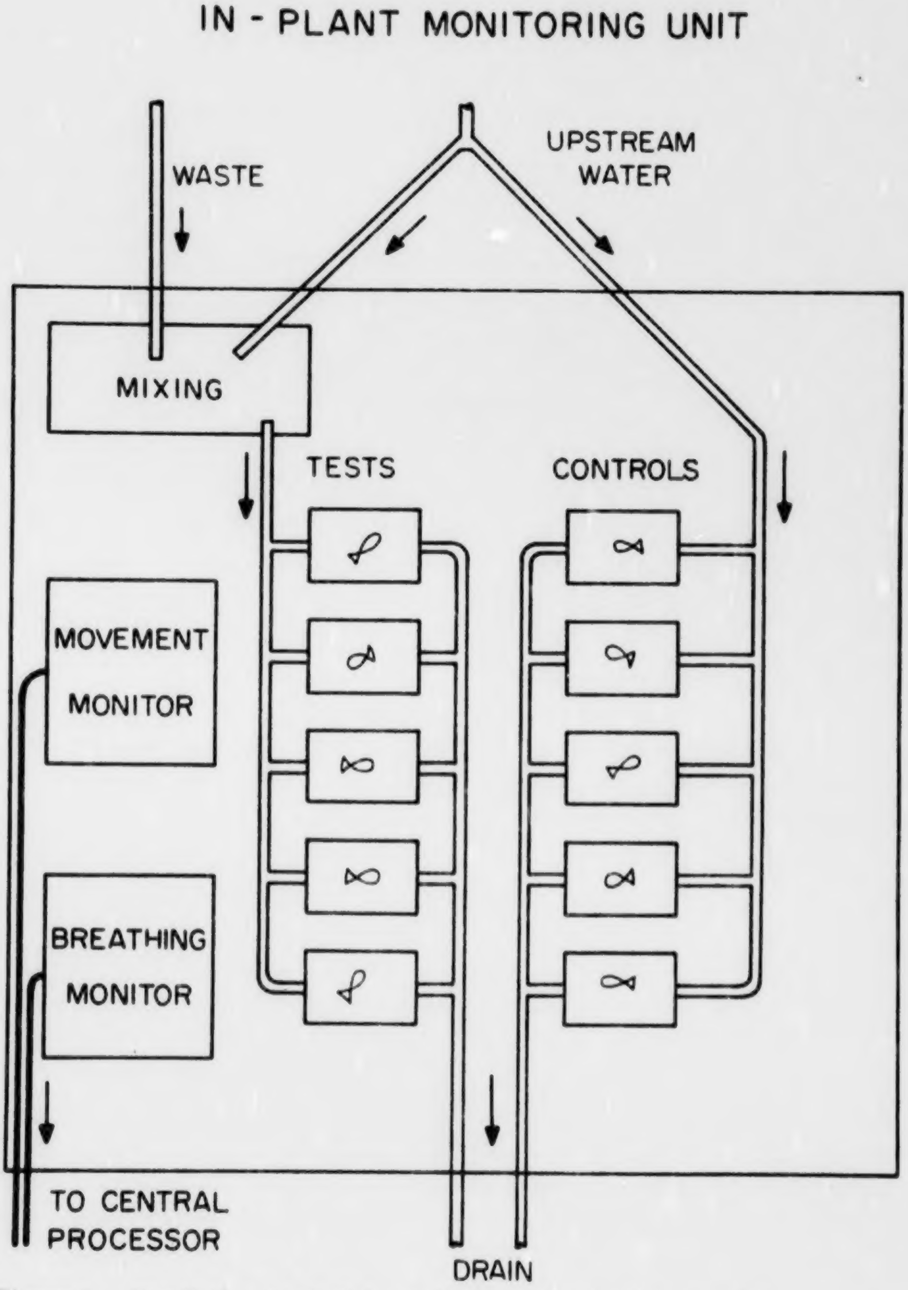

IN - PLANT MONITORING UNIT
WASTE
UPSTREAM WATER
MIXING
TESTS
CONTROLS
MOVEMENT MONITOR
BREATHING MONITOR
TO CENTRAL PROCESSOR
DRAIN

Figure 4. Detail of a single fish monitoring unit, showing how the experimental fish are exposed to waste diluted with upstream water and the control fish are exposed to upstream water alone.

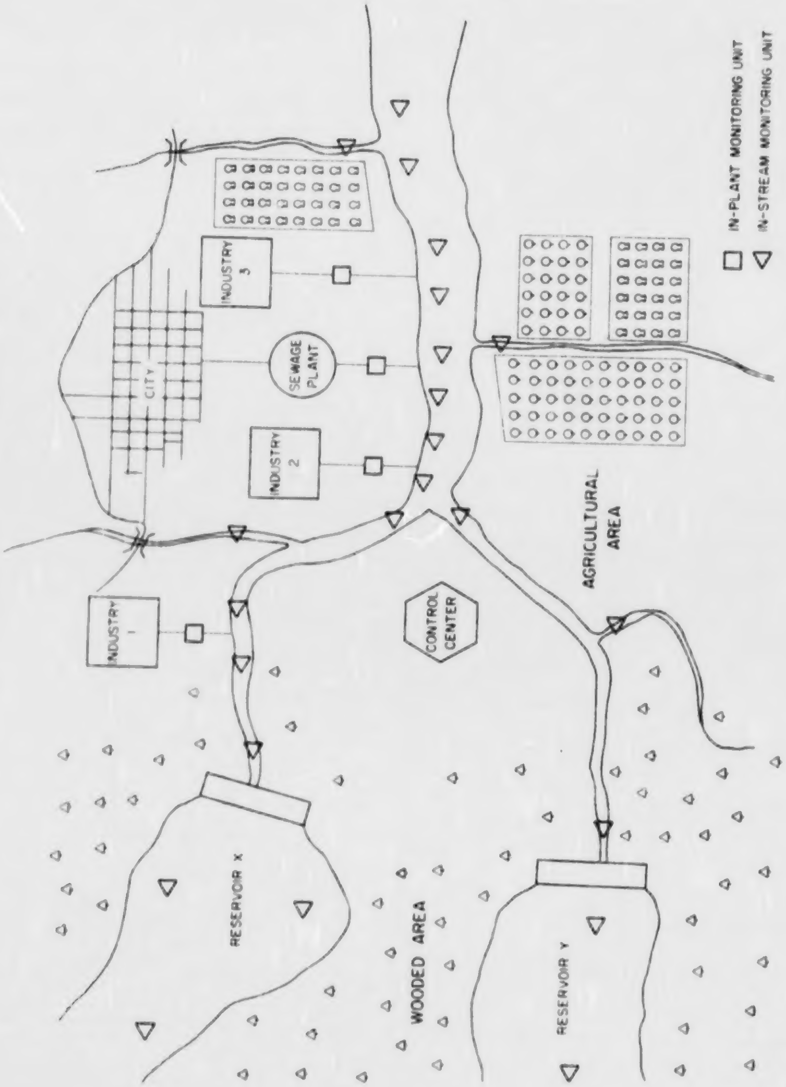

MIXED INDUSTRIAL AND AGRICULTURAL AREA
CITY
SEWAGE PLANT
INDUSTRY 3
INDUSTRY 2
INDUSTRY 1
CONTROL CENTER
AGRICULTURAL AREA
WOODED AREA
RESERVOIR X
RESERVOIR Y
□ IN-PLANT MONITORING UNIT
▽ IN-STREAM MONITORING UNIT

Figure 5. Use of in-plant monitoring units in a river management system.

AQUATIC ORGANISMS OF SIGNIFICANCE IN POLLUTION SURVEYS

I INTRODUCTION

A Any organism encountered in a survey is of significance. Our problem is thus not to determine which are of significance but rather to decide "what is the significance of each?"

B The first step in interpretation is recognition. "The first exercise in ecology is systematics."

C Recognition implies identification and an understanding of general relationships (systematics). The following outline will thus review the general relationships of living (as contrasted to fossil) organisms and briefly describe the various types.

D The species problem

1 Necessity of identifying species

Studies of the ecology of any habitat require the identification of the organisms found in it. One cannot come up with definitive evaluations of stress on the biota of a system unless we can say what species constitute the biota. Species vary in their responses to the impact of the environment.

2 Solutions to the problem

a Evasion

Treat the ecosystem as a "black box"--a unit--while ignoring the constitution of the system. This may produce some broad generalizations and will certainly yield more questions than answers.

b Compromise

Work only with those taxonomic categories with which one has the competence to deal. Describe the biotic component as a taxocenosis limited to one or two numerically dominant taxonomic categories, bearing in mind that numerically taxa which are ignored may be very important to the ecology of the ecosystem.

c Comprehensive description

Attempt a comprehensive description of the biota. No one can claim competence to deal with more than one or two groups. The cooperation of experts must be obtained. The Smithsonian Institution has a clearinghouse for this sort of thing.[1] Lists of expert taxonomists can be obtained. There will be none for some groups. Also collaboration is time consuming.

3 Fungi: extracellular digestion (enzymes secreted externally.) Food material then taken in through cell membrane where it is metabolized and reduced to the mineral condition. Ecologically known as REDUCERS.

E Each of these groups includes simple, single-celled representatives, persisting at lower levels on the evolutionary stems of the higher organisms.

1 These groups span the gaps between the higher kingdoms with a multitude of transitional forms. They are collectively called the PROTISTA.

2 Within the protista, two principal sub-groups can be defined on the basis of relative complexity of structure.

a The bacteria and blue-green algae, lacking a nuclear membrane may be considered as the lower protista (or Monera).

b The single-celled algae and protozoa are best referred to as the higher protista.

F Distributed throughout these groups will be found most of the traditional "phyla" of classic biology.

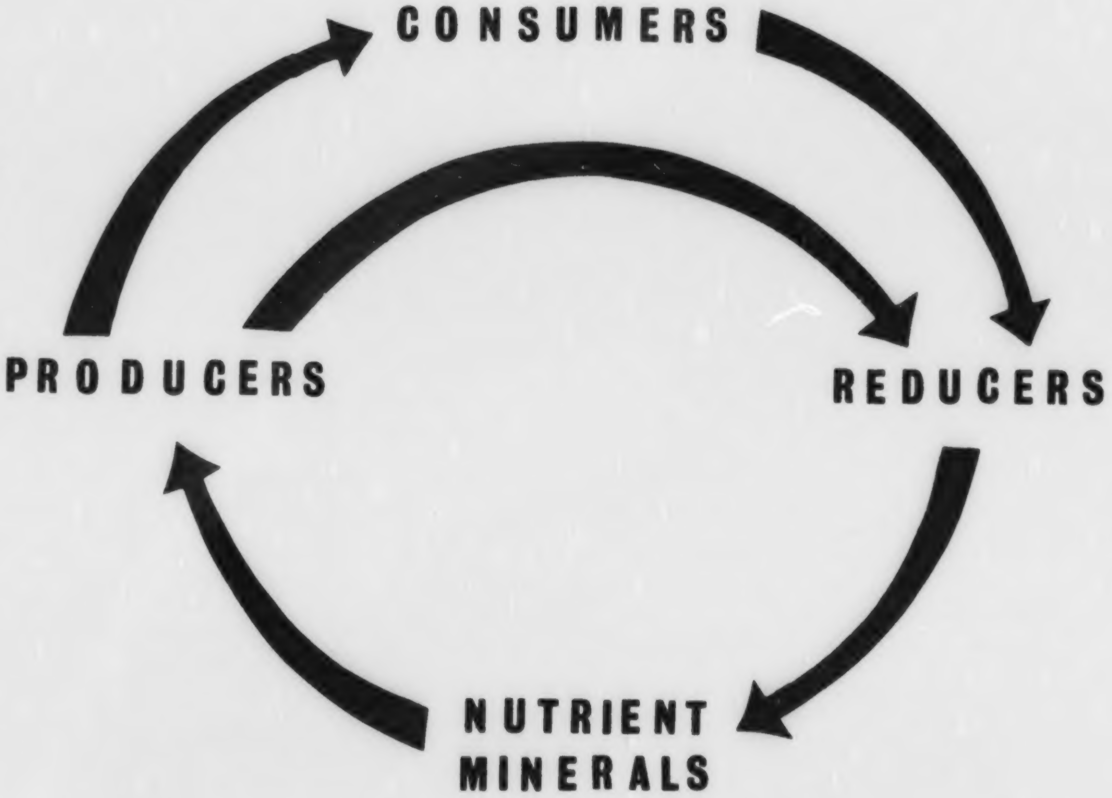

CONSUMERS
PRODUCERS
REDUCERS
NUTRIENT MINERALS

Figure 1. BASIC CYCLES OF LIFE

II PLANTS

A The vascular plants are usually larger and possess roots, stems, and leaves.

1 Some types emerge above the surface (emersed).

2 Submersed typed typically do not extend to the surface.

3 Floating types may be rooted or free-floating.

B Algae generally smaller, more delicate, less complex in structure, possess chlorophyll like other green plants. For convenience the following artificial grouping is used in sanitary science:

1 "Blue-green algae" are typically small and lack an organized nucleus, pigments are dissolved in cell sap. Structure very simple.

2 "Pigmented flagellates" possess nuclei, chloroplasts, flagellae and a red eye spot. This is an artificial group containing several remotely related organisms, may be green, red, brown, etc.

3 "Diatoms" have "pillbox" structure of SiO_2 - may move. Extremely common. Many minute in size, but colonial forms may produce hair-like filaments. Golden brown in color.

4 "Non-motile green algae" have no locomotor structure or ability in mature condition. Another artificial group.

a Unicellular representatives may be extremely small.

b Multicellular forms may produce great floating mats of material.

III FUNGI

Lack chlorophyll and consequently most are dependent on other organisms. They secrete extracellular enzymes and reduce complex organic material to simple compounds which they can absorb directly through the cell wall.

A Schizomycetes or bacteria are typically very small and do not have an organized nucleus.

1 Autotrophic bacteria utilize basic food materials from inorganic substrates. They may be photo-synthetic or chemosynthetic.

2 Heterotrophic bacteria are most common. The require organic material on which to feed.

B "True fungi" usually exhibit hyphae as the basis of structure.

IV ANIMALS

A Lack chlorophyll and consequently feed on or consume other organisms. Typically ingest and digest their food.

B The Animal Phyla

1 PROTOZOA are single celled organisms; many resembling algae but lacking chlorophyll (cf: illustration in "Oxygen" lecture).

2 PORIFERA are the sponges; both marine and freshwater representatives.

3 CNIDARIA (= COELENTERATA) include corals, marine and freshwater jelly fishes, marine and freshwater hydroids.

4 PLATYHELMINTHES are the flat worms such as tape worms, flukes and Planeria.

5 NEMATHELMINTHES are the round worms and include both free-living forms and many dangerous parasites.

6 ROTIFERS are multicellular microscopic predators.

7 BRYOZOA are small colonial sessile forms, marine or freshwater.

8 MOLLUSCA include snails and slugs, clams, mussels and oysters, squids, and octopi.

9 BRACHIOPODS are bivalved marine organisms usually observed as fossils.

10 ANNELIDS are the segmented worms such as earthworms, sludge worms and many marine species.

11 ECHINODERMS include starfish, sea urchins and brittle stars. They are exclusively marine.

12 CTENOPHORES, or comb jellies, are delicate jelly-like marine organisms.

13 ARTHROPODA, the largest of all animal phyla. They have jointed appendages and a chitinous exoskelton.

a CRUSTACEA are divided into a cephalothoraz and abdomen, and have many pairs of appendages, including paired antennae.

1) CLADOCERA include Daphina a common freshwater microcrustacean; swim by means of branched antennae.

2) ANOSTRACA (=PHYLLOPODS) are the fairy shrimps, given to eruptive appearances in temporary pools.

3) COPEPODES are marine and freshwater microcrustacea--swim by means of unbranced antennae.

4) OSTRACODS are like microscopic "clams with legs."

5) ISOPODS are dorsoventrally compressed; called sowbugs. Terrestrial and aquatic, marine and freshwater.

6) AMPHIPODA - known as scuds, laterally compressed. Marine and freshwater.

7) DECAPODA - crabs, shrimp, crayfish, lobsters, etc. Marine and freshwater.

b INSECTA - body divided into head, thorax and abdomen; 3 paris of legs; adults typically with 2 pairs of wings and one pair of antennae. No common marine species. Nine of the twenty-odd orders include species with freshwater-inhabiting stages in their life history as follows:

1) DIPTERA - two-winged flies

2) COLEOPTERA - beetles

3) EPHEMEROPTERA - may flies

4) TRICHOPTERA - caddis flies

5) PLECOPTERA - stone flies

6) ODONATA - dragon flies and damsel flies

7) NEUROPTERA - alder flies, Dobson flies and fish flies.

8) HEMIPTERA - true bugs, sucking insects such as water striders, electric light bugs and water boatman

9) LEPIDOPTERA - butterflies and moths, includes a few freshwater moths

c ARACHINIDA - body divided into cephalothorax and abdomen; 4 pairs of legs - spiders, scorpions, ticks and mites. Few aquatic representatives except for the freshwater mites and tardigrades.

C CHORDATA

1 PROTOCHORDATES - primitive marine forms such as acorn worms, sea squirts and lancelets

2 VERTEBRATES - all animals which have a backbone

a PICES or fishes: including such forms as sharks and rays, lampreys, and higher fishes; both marine and freshwater b AMPHIBIA - frogs, toads, and salamanders - marine species rare.

c REPTILA - snakes, lizards and turtles.

d MAMMALS - whales and other warm-blooded vertebrates with hair.

e AVES - birds - warm-blooded vertebrates with feathers.

REFERENCE

Whittaker, R. H. New Concepts of Kingdoms of Organisms. Science 163:150-160. 1969.

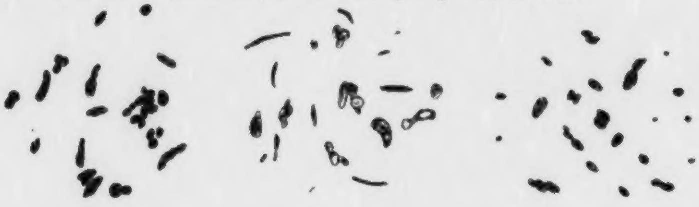
This outline was prepared by H. W. Jackson, formerly Chief Biologist, National Training Center, Water Program Operations, EPA, Cincinnati, OH 45268, and revised by Ralph M. Sinclair, Aquatic Biologist, National Training Center.

Descriptors:
Aquatic Life; Systematics

FUNGI

3/4

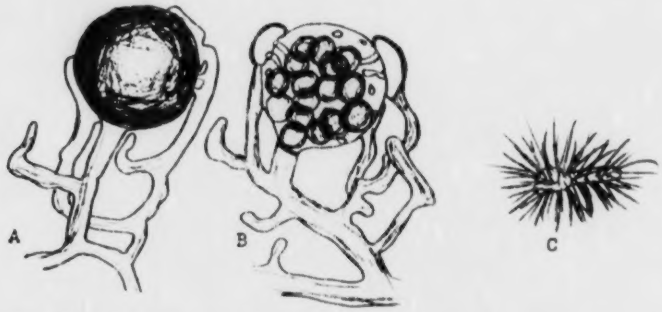
Schizomycetes - Bacteria, free living representatives



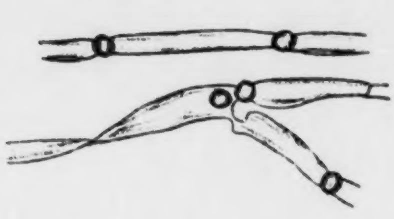
Aerobacter aerogenes

Rhizobium radicicola

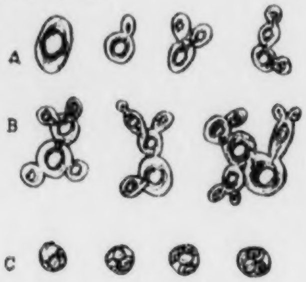
Azotobacter



Phycomycetes - Saprolegnia; A, detail of immature reproductive stages; B, mature oogonium and antheridia, with eggs and fertilization tubes; C, dead tadpole with growth of S.



Phycomycete - Leptomitus, this genus includes pollution tollerant species.



Ascomycete - Saccharomyces, a yeast including poll. tollerant species. A, single cell; B, budding; C, ascospore formation.

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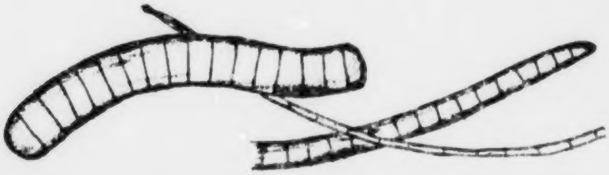
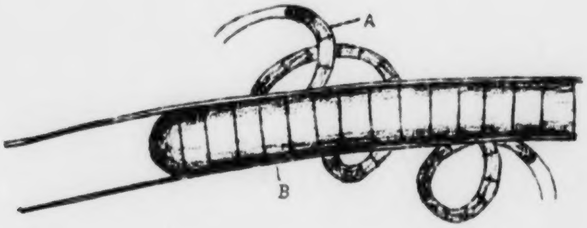
PLATE I

BLUE GREEN ALGAE

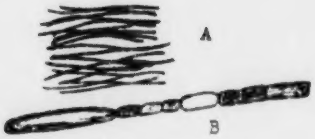
3/4



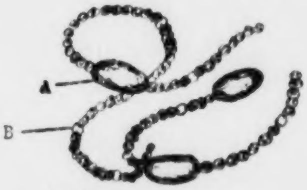
Oscillatoria spp., filaments (trichomes) range from .6 to over 60µ in diameter. Ubiquitous, pollution tollerant.



Lyngbia spp., similar to Oscillatoria but has a sheath. A, Lyngbia contorta, reported to be generally intollerant of pollution; B, L. birgei.



Aphanizomenon flos-aquae
A, colony; B, filament



Anabaena flos-aquae
A, akinete; B, heterocyst

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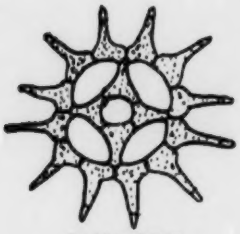
PLATE II

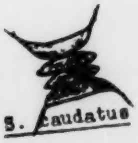
1/67

NON-MOTILE GREEN ALGAE: COCCOID
(CHLOROPHYCEAE)

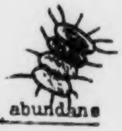


Pediastrum

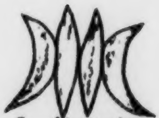
Species of the Genus Scenedesmus



S. caudatus

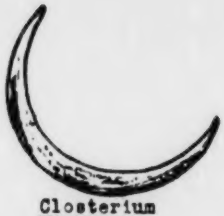


S. abundans

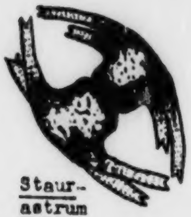


S. dimorphus

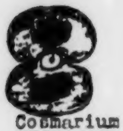
Desmids



Closterium



Staur-
astrum



Cosmarium

PLATE III

11/3 NON-MOTILE GREEN ALGAE: FILAMENTOUS

(Chlorophyceae)

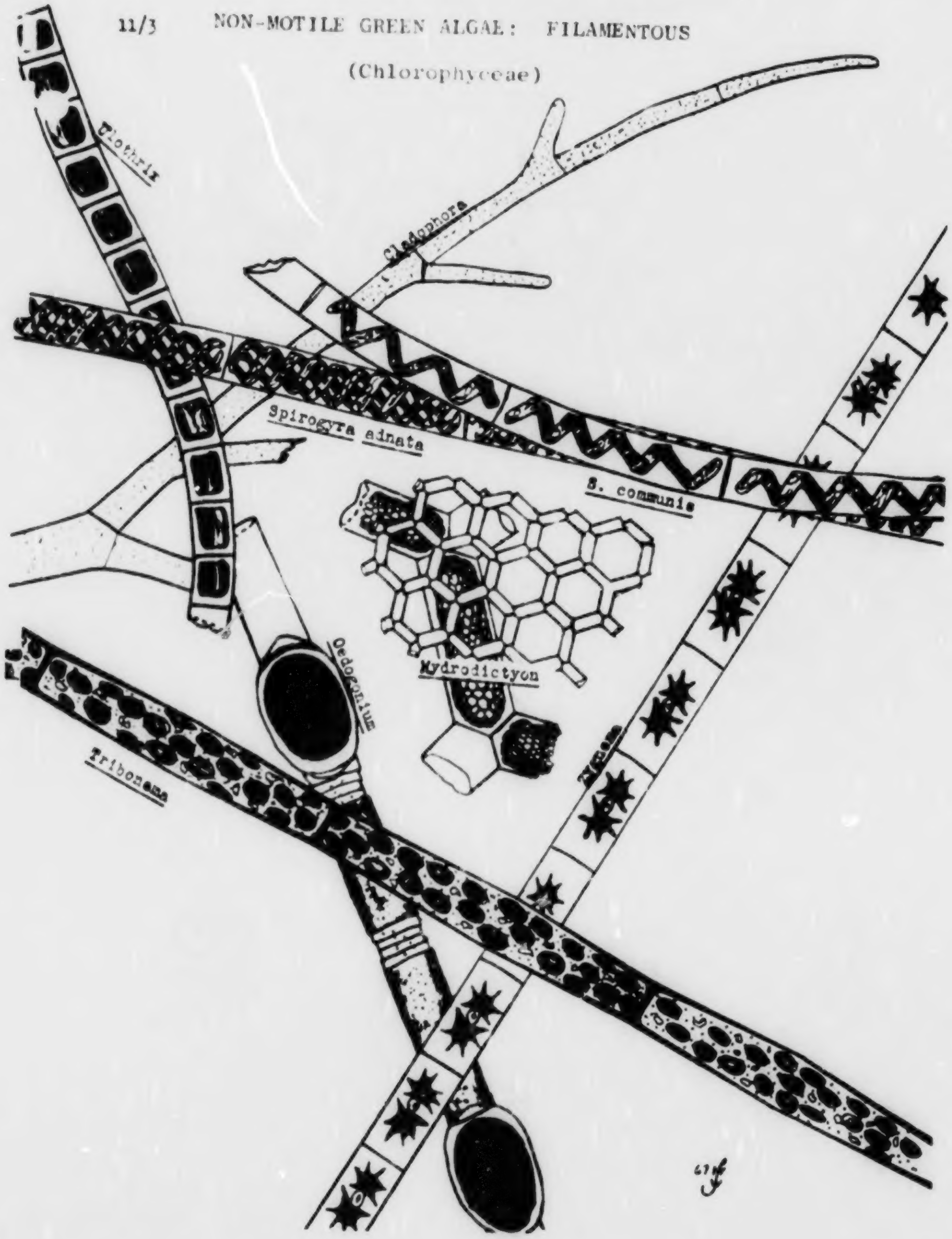

Ulothrix
Cladophora
Spirogyra adnata
S. communis
Hydrodictyon
Oedogonium
Tribonema
Zygnema

PLATE IV

30-9

PIGMENTED FLAGELLATES

3/4

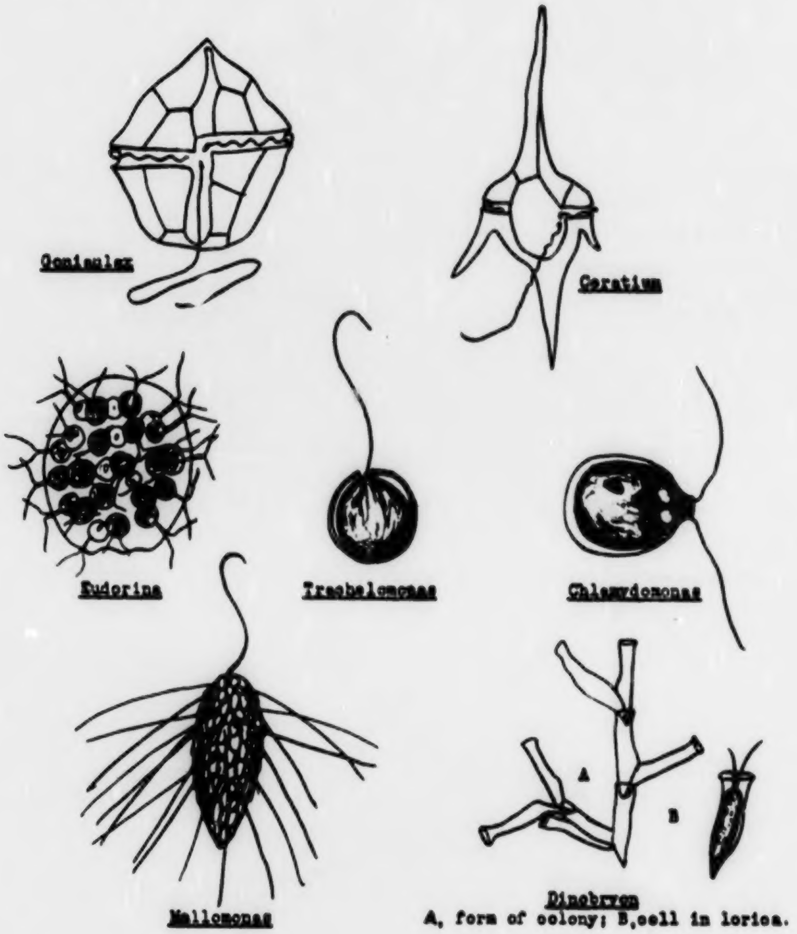

Gonyaulax
Ceratium
Eudorina
Trachelomonas
Chlamydomonas
Mallomonas
A
B
Dinobryon
A, form of colony; B, cell in lorica.

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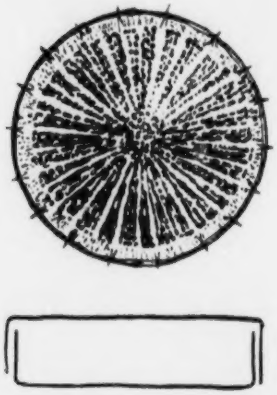
PLATE V

DIATOMS

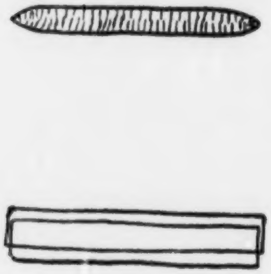
3/4



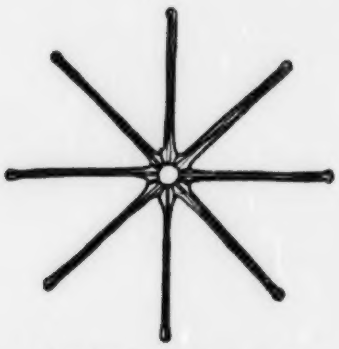
A discoid or central diatom such as Stephanodiscus

Valve views

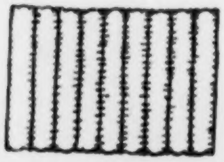
Girdle views, stylized to show basic diatom structure.



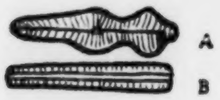
A pennate or navicular diatom such as Fragillaria



A colony of Asterionella (girdle views)



A colony of Fragillaria (girdle views)



Gomphonema A, valve view; B, girdle view.

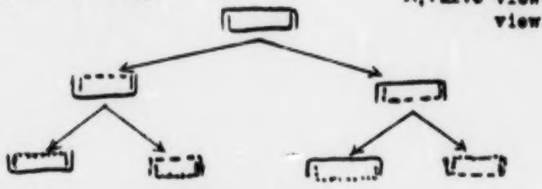


Diagram showing progressive diminution in the size of certain frustules through successive cell generations of a diatom.

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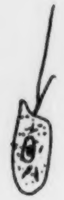
PLATE VI

.30 //

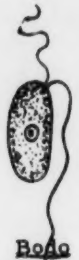
3/4

FREE LIVING PROTOZOA

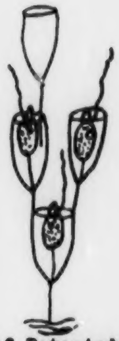
I. Flagellated Protozoa, Class Mastigophora



Anthophysis
Pollution tolerant
6 µ

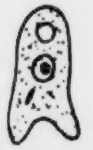


Bodo
Pollution tolerant
19 µ

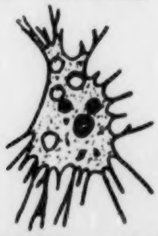


Colony of **Poteriodendron**
Pollution tolerant , 35 µ

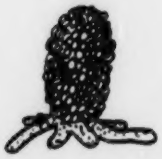
II. Ameboid Protozoa, Class Sarcodina



Dimastigamoeba
Pollution tolerant
10-50 µ

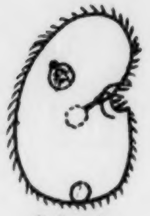


Nuclearia, reported to be intolerant of pollution, 45 µ.

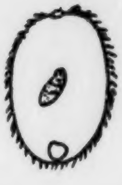


Difflugia
Pollution tolerant
60-500 µ

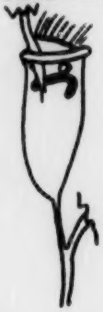
III. Ciliated Protozoa, Class Ciliophora



Colpoda
Pollution tolerant
20-120 µ



Holophrya, reported to be intolerant of pollution, 35 µ



Epistylis, pollution tolerant Colonies often macroscopic.

PLATE VII

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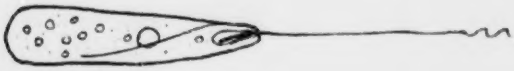
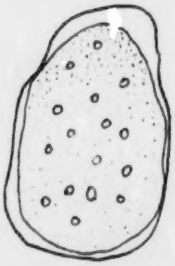
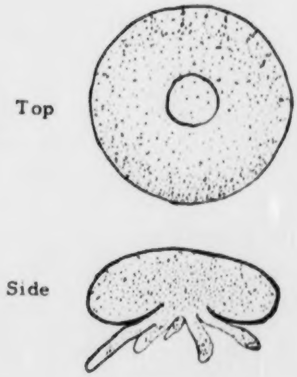
PLANKTONIC PROTOZOA



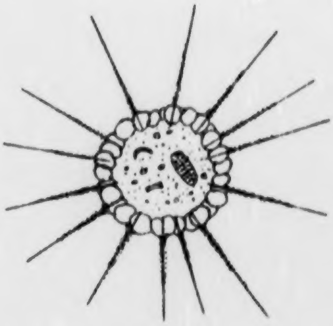
Peranema trichophorum



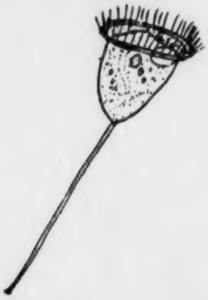
Chaos



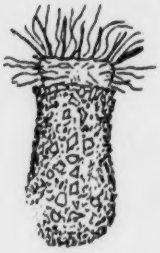
Arcella vulgaris



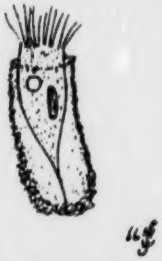
Actinosphaerium



Vorticella



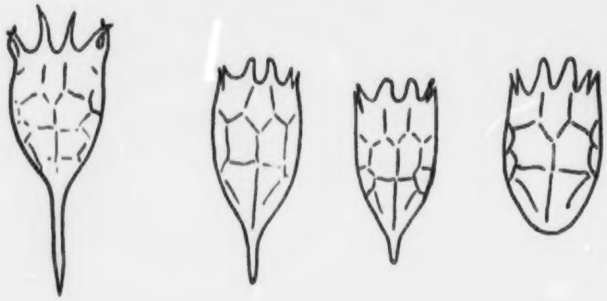
Codonella cratera



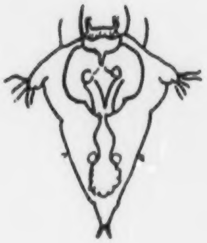
Tintinnidium fluviatle

PLATE VIII

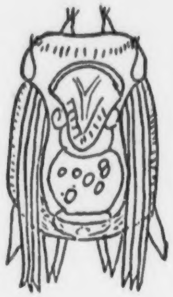
PLANKTONIC ROTIFERS



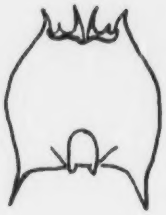
Various Forms of *Keratella cochlearis*



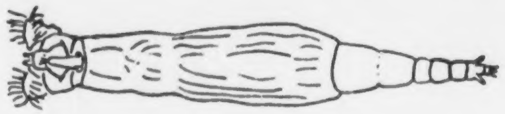
Synchaeta pectinata



Polygarthra vulgaris



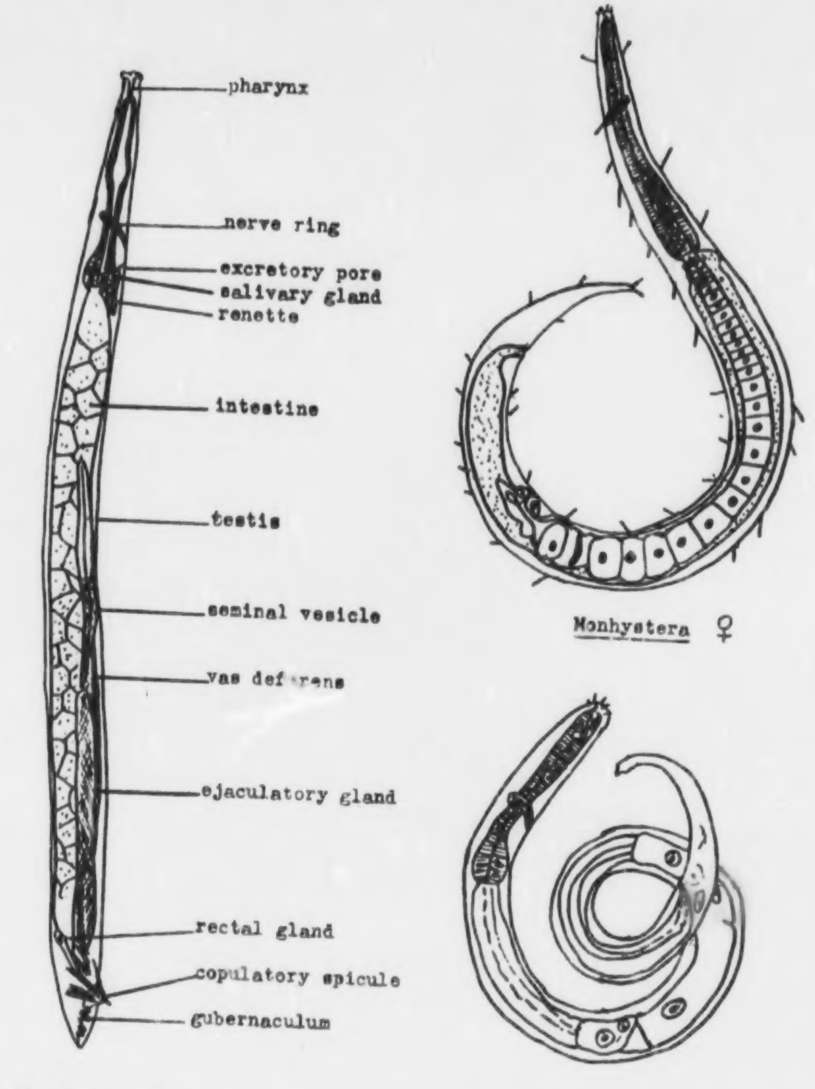
Brachionus quadridentata



Rotaria sp

PLATE IX

FREE LIVING NEMATHELMINTHES, OR ROUND WORMS


pharynx
nerve ring
excretory pore
salivary gland
renette
intestine
testis
seminal vesicle
vas deferens
ejaculatory gland
rectal gland
copulatory spicule
gubernaculum

Monhystera ♀

Rhabditis ♂

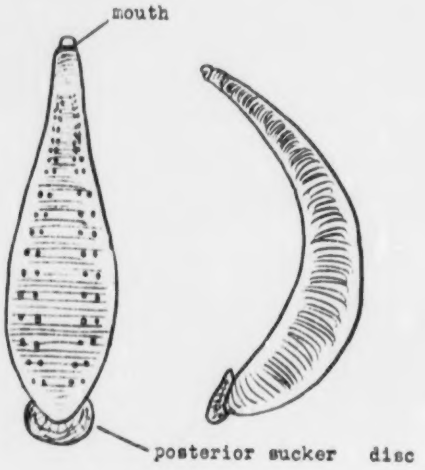
Achromadora ♀

PLATE X

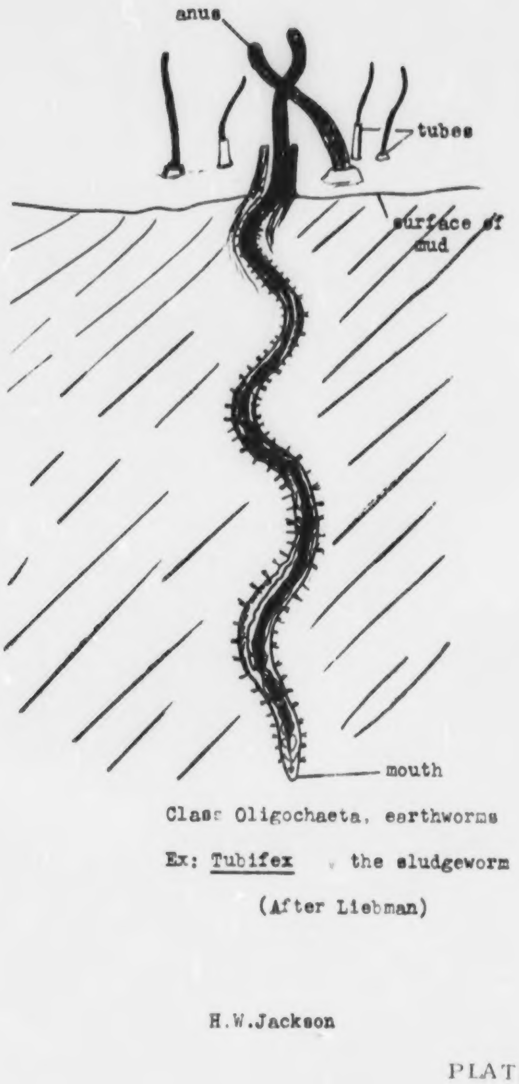
30 - 15

FRESH WATER ANNELID WORMS

Phylum Annelida


mouth
posterior sucker disc

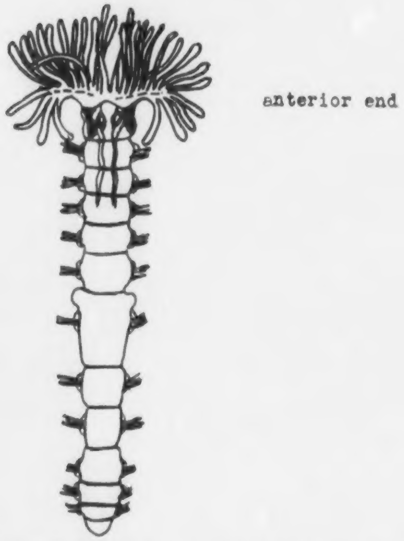
Class Hirudinea, leeches
(After Hegner)


anus
tubes
surface of mud
mouth

Class Oligochaeta, earthworms

Ex: Tubifex , the sludgeworm

(After Liebman)


anterior end

Class Polychaeta , polychaet worms

Ex: Manayunkia, a minute, rare, tube building worm.
(After Leidy)

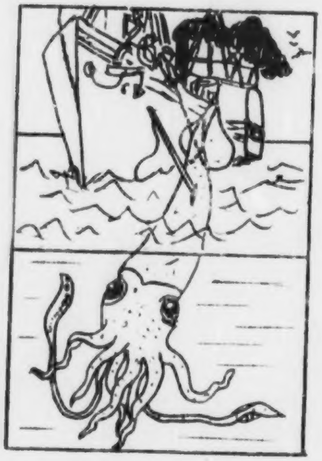
H.W.Jackson

PLATE XI

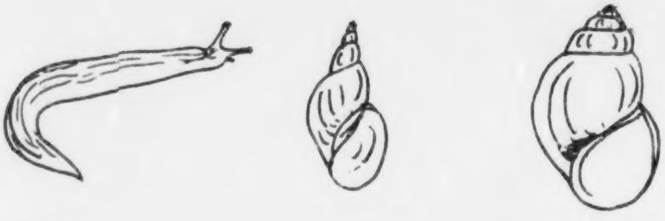
30.16

SOME MOLLUSCAN TYPES

Class: Cephalopoda:
Squids, octopus,
cuttlefish.



Exclusively marine. The giant squid shown was captured in the Atlantic in the early ninteenth century. (After Hegner)

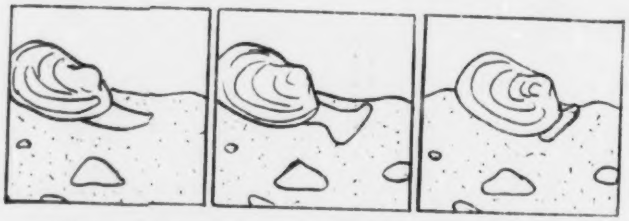


Limax, a slug

Lymnaea an air breathing snail

Campeloma a water breathing snail

Class: Gastropoda; snails and slugs. (After Buchsbaum)



Class: Pelecypoda; clams, mussels, oysters.

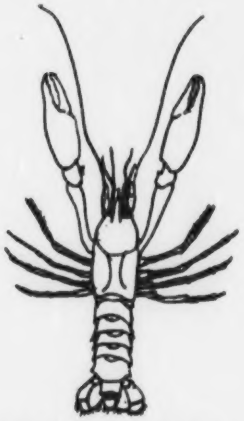
Locomotion of a freshwater clam, showing how foot is extended, the tip expanded, and the animal pulled along to its own anchor. (After Buchsbaum)

H.W.Jackson

PLATE XII

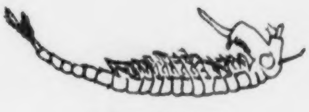
Class CRUSTACEA

3/4

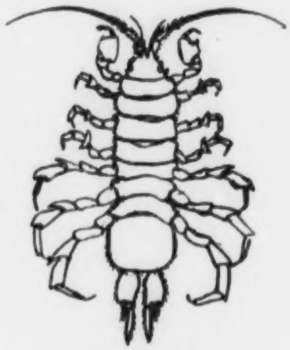


Crayfish, or crawdad; Cambarus, Order Decapoda

10-20 cm

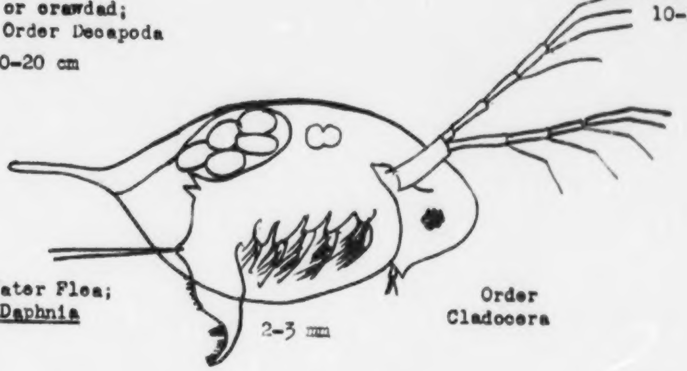


Fairy Shrimp; Eubranchipus, Order Phyllopoda 20-25 mm



Sow Bug; Asellus, Order Isopoda

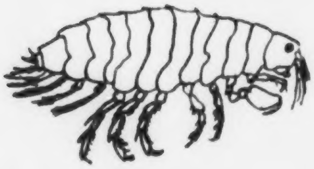
10-20 mm



Water Flea; Daphnia

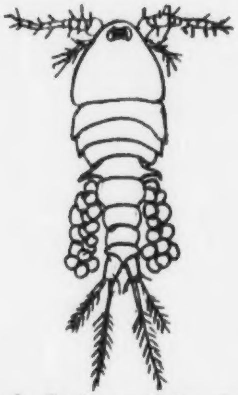
2-3 mm

Order Cladocera



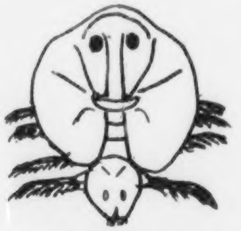
Scud; Hyalella Order Amphipoda

10-15 mm



Copepod; Cyclops, Order Copepoda

2-3 mm



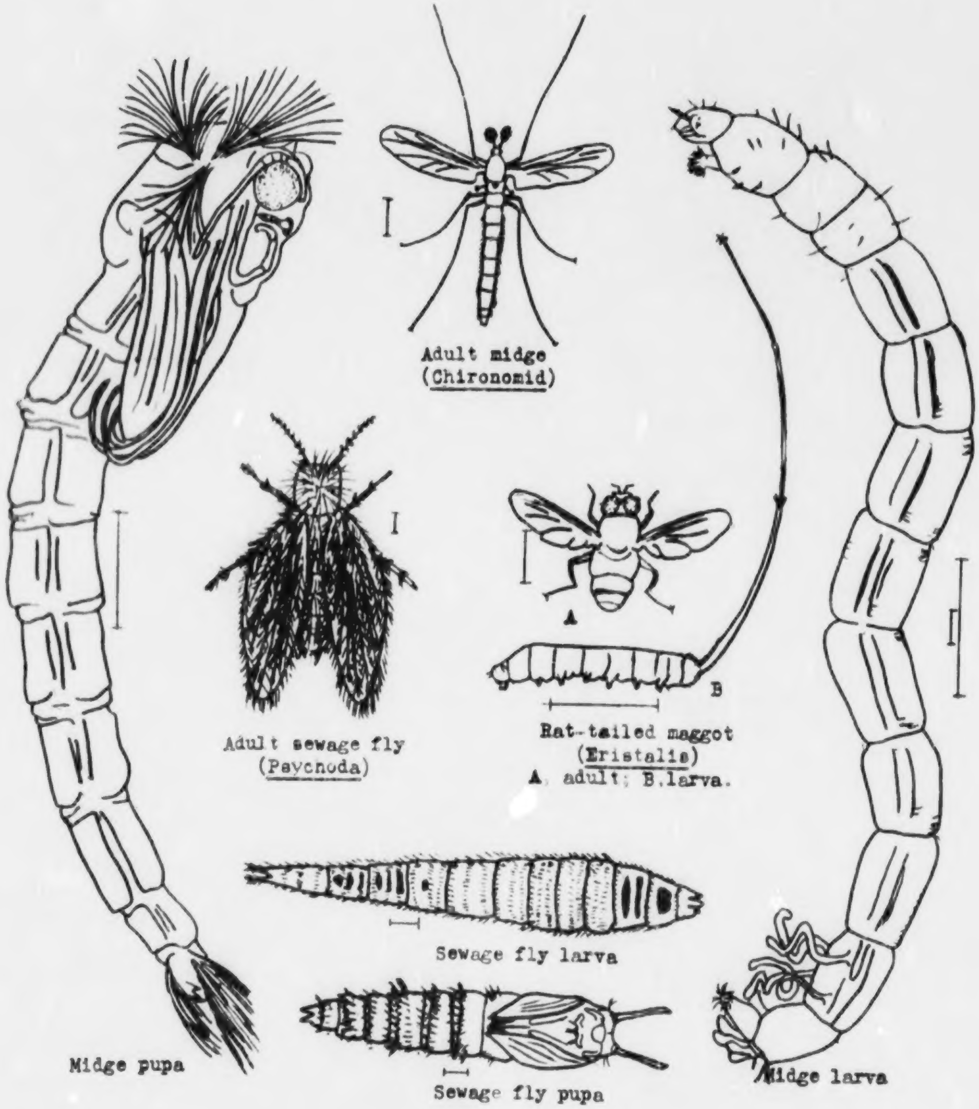
Fish Louse, Argulus; a parasitic Copepod

5-6 mm

H.W.Jackson

PLATE XIII

Two Winged Flies
Order DIPTERA

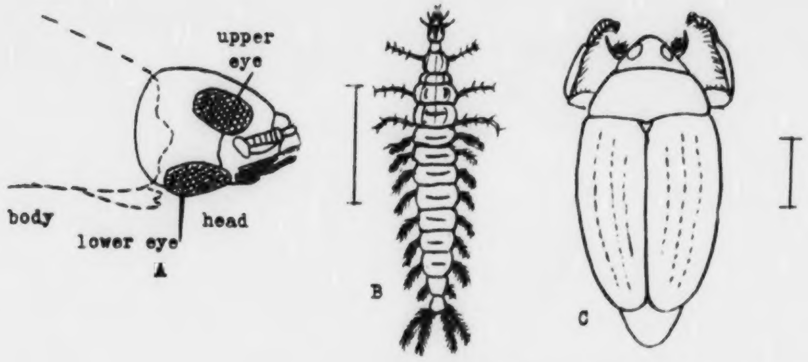

Adult midge
(Chironomid)
Adult sewage fly
(Psychoda)
Rat-tailed maggot
(Eristalis)
A, adult; B, larva.
A
B
Sewage fly larva
Midge pupa
Sewage fly pupa
Midge larva

H.W.Jackson
After various authors

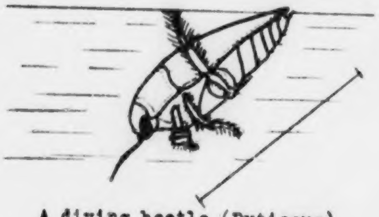
PLATE XIV

30.19

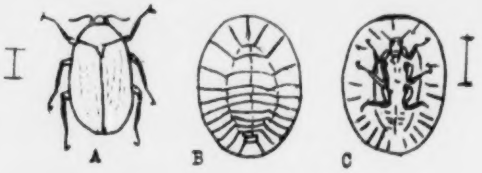
Beetles
Order COLEOPTERA



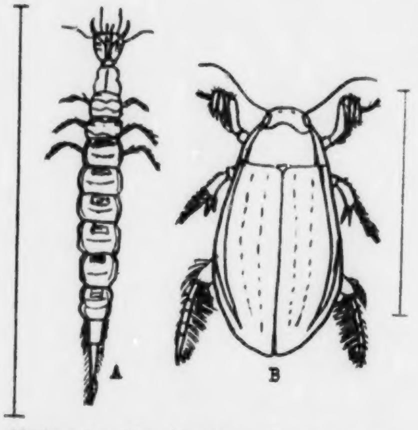
Whirligig beetle (Gyrinus) A. Side view of head of adult showing divided eye; B. Larva; C. Adult. Carnivorous.



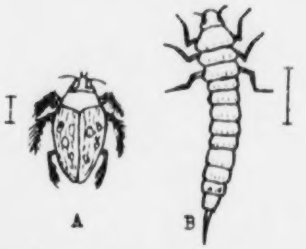
A diving beetle (Dytiscus) taking air at the surface.



The riffle beetle (Psephenus); A. adult; B. dorsal side of larva; C. ventral side of larva. Predominantly herbivorous.



A diving beetle (Cybister). The diving beetles include some of the largest and most varasious of aquatic insects. A. larva; B.adult.



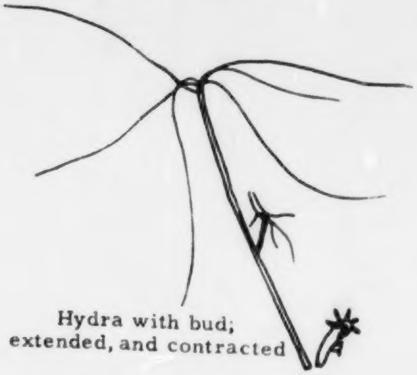
Crawling water beetle; A;adult; B.larva. Predominantly herbivorous.

H.W.Jackson. After Needham, Pennak, Morgan, and others.

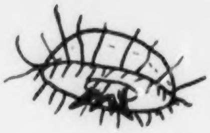
PLATE XV

MINOR PHYLA

Phylum Coelenterata

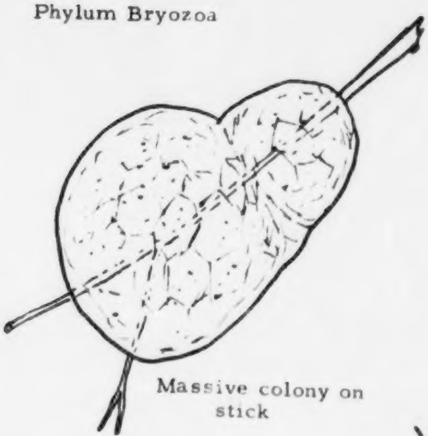


Hydra with bud; extended, and contracted

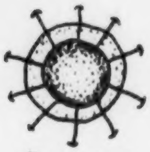


Medusa of Craspedacusta

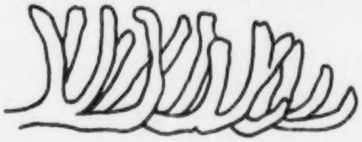
Phylum Bryozoa



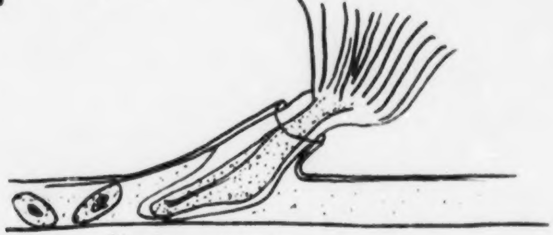
Massive colony on stick



Statoblast



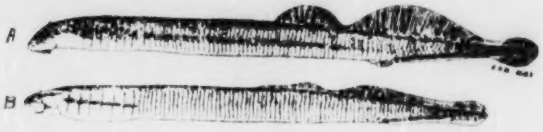
Creeping colony on rock



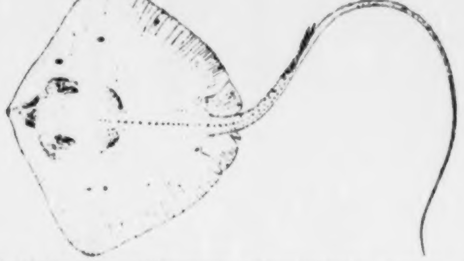
Single zooid, young statoblasts in tube

PLATE XVI

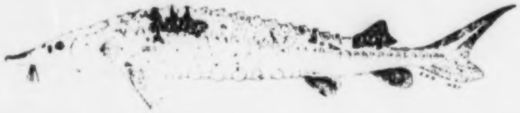
SOME PRIMITIVE FISHES



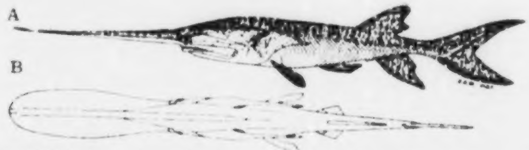
Class Agnatha, jawless fishes (lampreys and hagfishes) - Family PETROMYZONTIDAE, the lampreys. Lampetra aepyptera, the Brook Lamprey A: adult, B: larva (enlarged)



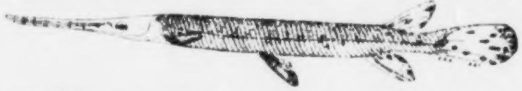
Class Chondrichthyes - cartilagenous fishes (sharks, skates, rays) Family DASYATIDAE - stingrays. Dasyatis centroura, the Roughtail Stingray



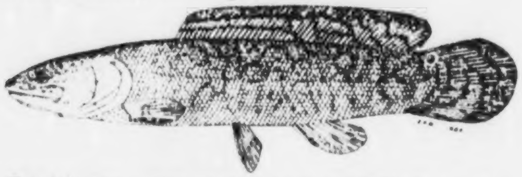
Class Osteichthyes - bony fishes - Family ACIPENSERIDAE, sturgeon. Acipenser fulvescens, the Lake Sturgeon



Class Osteichthyes - bony fishes - Family POLYODONTIDAE, the paddlefishes. Polyodon spathula, the Paddlefish. A:side view B:top view



Class Osteichthyes - bony fishes - Family LEPISOSTEIDAE - gars Lepisosteus osseus, the Longnose Gar



Class Osteichthyes - bony fishes - Family AMIIDAE, bowfins Amia calva, the Bowfin

Reproduced with permission; Trautman, 1957.

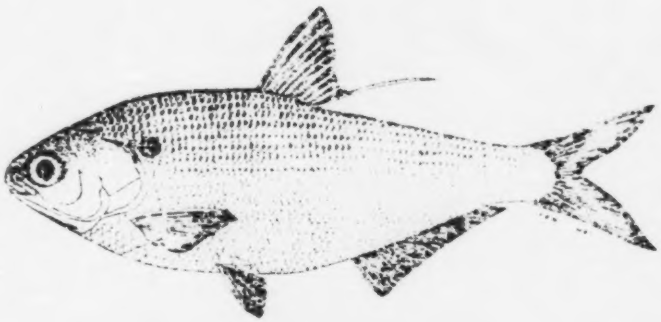
BI. AQ. pl. 91. 6. 60

PLATE XVII

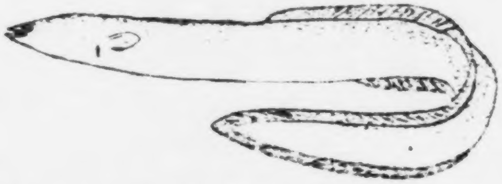
30.22

30-23

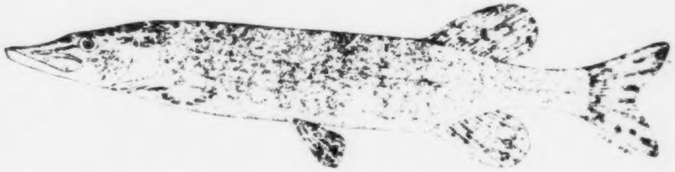
TYPES OF BONY FISHES



Family CLUPEIDAE - herrings
Dorosoma cepedianum - the eastern gizzard shad



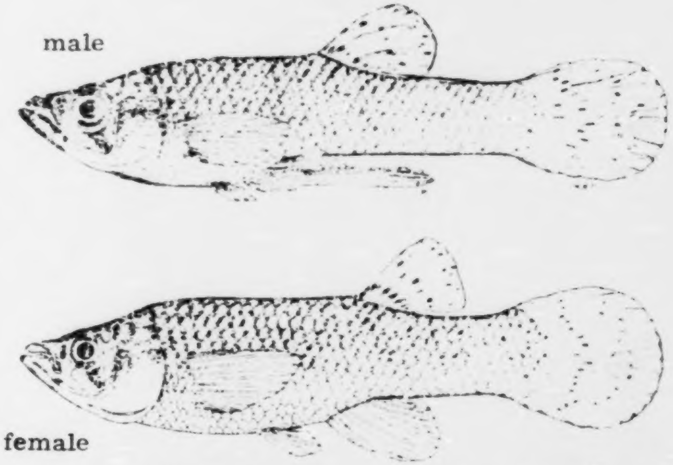
Family ANGUILLEDAE - freshwater eels
Anguilla rostrata - the American eel



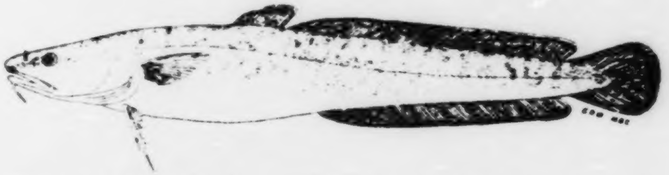
Family ESOCIDAE - pikes
Esox lucius - the northern pike

Reproduced with permission; Trautman, 1957.

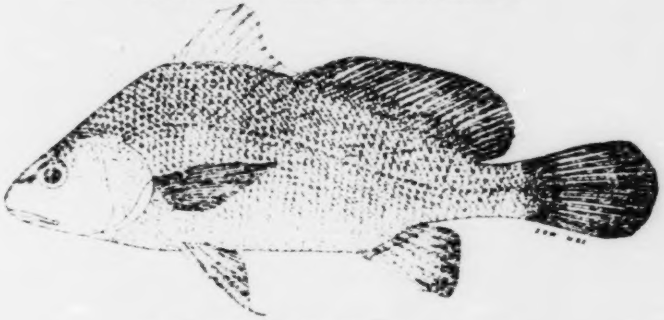
BI. AQ. pl. 9m. 6. 60



Family POECILIIDAE - livebearers
Gambusia affinis - the mosquitofish



Family GADDIDAE - codfishes, hakes, haddock, burbot
Lota lota - the eastern burbot



Family SCIAENIDAE - drums
Aplodinotus grunniens - the freshwater drum

PLATE XVIII

ANALYTICAL QUALITY CONTROL IN EPA, WPO

I NEED FOR QUALITY CONTROL

A Importance of Laboratory Data

1 Water quality standards

The establishing, implementation, and enforcement of water quality standards requires very accurate information on existing water quality in all the major surface waters of the United States. Determination of compliance with the standards, and the economic, legal, and social actions associated with pollution abatement, must rest on reliable data.

2 Waste treatment

The efficiency of waste treatment systems must be accurately determined by laboratory data, in order to evaluate treatment processes and waste discharges.

3 Research

In most research investigations, laboratory data defines the information sought, the success or failure of alternative pathways, and often describes the final answer obtained.

B Reliability Requirements

1 Specificity

To be useful, data on chemical constituents must clearly indicate what is being measured, including the physical state and the chemical structure.

2 Accuracy

The accuracy required is defined by the final use of the data, and is directly related to the interpretation of the values reported in terms of water quality or waste constituent concentration. For proper interpretation, the limits of accuracy must also be known.

3 Precision

The analyst must know the precision of his methods, in order to determine when the procedure is in control. The precision establishes the confidence one can have in a single reported result.

II ANALYTICAL METHODS SELECTION

A Available Sources of Methods

1 Standard Methods for the Examination of Water and Wastewater

2 ASTM Standards, Part 23, Water; Atmospheric Analysis

3 Current literature

4 Methods research in WPO laboratories

B Selection Process

The sources listed above are reviewed regularly and preliminary selection is made by the staff of the Methods Development and Quality Assurance Laboratory. After collaborative testing, the final selection is reviewed and approved by WPO Regional AQC Coordinators or by Advisory Committees.

C Status of WPO Methods

The analytical procedures adopted are used in all WPO laboratories, except where the analyst has determined that another method must be used because of unusual interferences or special data requirements. The methods are also used by contractors providing services to WPO

D Publications

WPO methods are published by the Methods Development & Quality Assurance Laboratory and distributed to Federal, State, and private laboratories through the Regional AQC Coordinators.

III METHODS AND PERFORMANCE EVALUATION

A Collaborative Testing

1 Purpose

To verify the specificity, sensitivity, precision, and accuracy of the adopted methods, round-robin testing is carried out through the cooperation of WPO and other laboratories. Statistical evaluation of test data establishes the reliability of the method in our laboratories and determines the need for improvement or further research.

2 Sample studies

Reference samples for a majority of the parameters used in determining water quality have been prepared or are planned. The studies are designed to test the method at the concentrations found in water and waste samples, in the presence of the interferences normally encountered.

B Performance Testing

The ability of the individual analyst to obtain a valid result is an important part of the quality control program. The reference sample studies are designed to evaluate the performance of the analyst, in comparison with others participating in the study. Thus the analyst, and his supervisor, can identify problems requiring tighter control or additional training.

IV INTRALABORATORY QUALITY CONTROL

A Elements of a Control Program

An adequate quality control program in a laboratory should include checks on:

1 Methods

2 Reagents

3 Instrumentation

4 Glassware

5 Daily performance (control charts)

6 Data handling and reporting

B Responsibilities for Quality Assurance

The basic responsibility for the quality of laboratory data rests with the analyst himself. A formal program in the laboratory, however, provides the laboratory director with a mechanism for evaluating the reliability of the data and maintaining satisfactory quality.

This outline was prepared by D. G. Ballinger, Director, Methods Development & Quality Assurance Research Laboratory EPA, NERC - Cincinnati

Descriptors: Chemical Analysis, Quality Control, Water Analysis

AUTOMATIC INSTRUMENTS FOR WATER QUALITY MEASUREMENTS

I NEED FOR CONTINUOUS MONITORING OF WATER QUALITY

Satisfactory evaluation of the quality of water depends upon the availability of adequate data. Such data must be not only as accurate as economically practical but must also be of sufficient quantity to support reliable conclusions. In general, the more information available, the more reliable will be the interpretations.

In the past, information on the chemical, physical, and bacteriological quality of most surface waters has been obtained by periodic stream surveys or infrequent "spot" analyses. Although raw water supplies are sampled daily, the data obtained are restricted to those tests of importance in water treatment and do not include other pollutional parameters or water resources not at present being used for water supply.

It is apparent that in many situations significant changes in water quality may occur often and abruptly. Seasonal changes in flow, the occurrence of unpredictable industrial discharges or spills, and the changes in flow from impoundments may alter the concentration of many of the substances of interest to the water user. In some cases these changes may occur within a few hours: for example, the diurnal fluctuations in dissolved oxygen and the salinity changes in tidal estuaries. Thus considerable advantage is gained if continuous monitoring of water quality can be accomplished. The use of manual sampling and standard laboratory analyses would be far too expensive; therefore a wide variety of automatic instruments have been developed.

II ELECTRICAL INSTRUMENTS

The diagram below illustrates a typical system for monitoring instruments which utilize electrical sensors. The sensor produces an electrical signal representative of concentration, the signal is converted, and amplified, then passed to the recorder to develop a permanent record of concentration levels. For a clear understanding of the operational characteristics, the parts of the system are discussed separately.

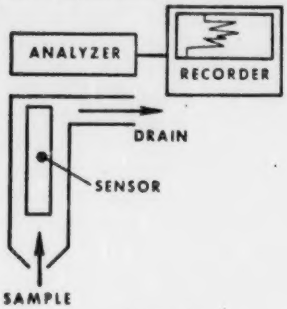


Figure 1

A Sensors

The sensor is the part of the system in contact with the sample. Sensing elements may be immersed directly in the stream, or placed in flow cells through which the sample is pumped. Both methods have advantages and limitations.

When the sensor is placed in the stream the determination is made "in situ." Therefore the sample is not affected by pumping, temperature changes, or time of travel through the instrument. Such an installation, however, presents certain problems. The sensing elements must be protected from floating debris and must be mounted so as to remain in a fixed position in spite of changes in velocity or direction of current. In bodies of water which fluctuate in surface level, such as impoundments, or estuaries, the relative depth of the sensor may change. In addition, frequent inspection of the sensing elements for attached growths or physical damage is difficult and apt to be neglected.

The use of the shore-based system, where the stream sample is pumped through flow cells within the instrument housing, is free from some of the difficulties mentioned previously. Inspection of the sensors is easy, cleaning is simplified, and replacement of sensing elements is readily accomplished. It should be recognized, however, that the precautions required for satisfactory mounting and protection of sensor units in the stream apply equally well to the pump intake. Further, it is essential that the sample being tested in the flow cell is truly representative of the stream water. If dissolved oxygen is included in the parameters, a submersible pump is required, to avoid cavitation and prevent suction removal of dissolved gases. The intake screen must be carefully designed, since a fine screen will quickly clog, while a coarse screen may permit floating material to enter the system.

Electrical sensors may be conductimetric, potentiometric, polarographic, or coulimetric. The sensor may directly measure a constituent or property of the sample or it may be used as an indicating mechanism in automatic titration.

B Analyzer-Amplifier

The function of the analyzer is to convert the signal from the sensor to a standard EMF, usually ranging from 0-50 millivolts. Often bridge circuits are employed. The analyzer must be rugged to avoid shock damage and the electronic circuitry must be stable over a wide range of environmental conditions. Considerable advantage is gained by the use of standard, readily available components. Provision should be made for a standard signal to permit internal checking of the circuits.

In most cases the signal must be amplified to provide sufficient voltage to drive a recorder. Amplification is generally built into the analyzer circuitry.

C Recorder

Although read-out meters can be used, a permanent record is desirable. Strip-chart recorders are the most popular even though they require line voltage. A slow chart speed is necessary, since observation periods of several days are common. Difficulties with pens are often encountered due to varying conditions of humidity and temperature. The use of pressure-sensitive paper may offer distinct advantage.

Circular charts have long been used but they have certain disadvantages. Unless the chart is inconveniently large the chart divisions are very close together making hourly fluctuations difficult to interpret. In addition, the circular chart does not lend itself to mechanical handling of data for computer processing. Chart drive by clock mechanism is possible, however, eliminating the need for power connection.

A preferable system for the recording of data is the use of digital output. Water quality instruments can be equipped with analog-to-digital converters which change the amplitude of the signal to a digital value. By the use of a punch-tape readout the data is recorded on paper tape rather than on a chart. The punched tape may then be transferred to a computer for data processing. Therefore, a monitor equipped with digital tape readout permits the recording of original signal and the performance of statistical computations without hand transcription.

When the Surveillance Program includes several automatic monitors on a river system , consideration should be given to a central data logging facility. By transmitting the data from the monitors directly to a small computer (by leased phone lines or radio), the information on water quality of the entire river basin is immediately available. The central facility also provides for the detection of unusual discharges which may affect downstream water users.

D Parameters Measured Electrically

The following water quality parameters can be measured by the use of electrodes:

Temperature
Conductivity
pH
Oxygen
Chloride
Residual chlorine
Oxidation-reduction potential

A prominent instrument manufacturer is now conducting research into the development of a number of glass electrodes specifically designed for the measurement of other ions. It appears that the list above may be considerably expanded in the future.

In most cases precision and accuracy is not as good as with standard laboratory tests, but the advantages of continuous recording outweigh the limitations in performance.

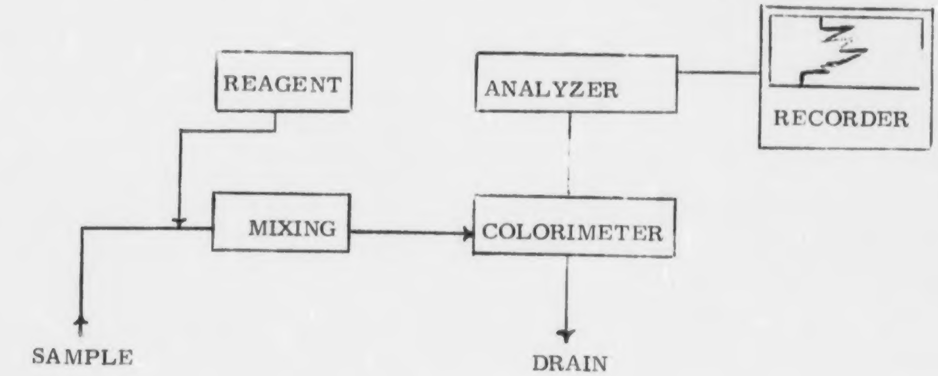
III PHOTOMETRIC INSTRUMENTS

Because of the importance of colorimetric analysis in the water laboratory, considerable attention has been given to the development of continuous monitoring instruments employing this principle. A typical system is shown in Figure 2.

It should be noted that the sample must be pumped through the instrument with the attendant problems described above.

A Measurement of Turbidity and Color

In the simplest photometric instrument, a property of the sample, such as turbidity or color, is measured directly. These relatively simple parameters, however, are rather difficult to determine. Turbidity measurements are affected by particle size and by the true color of the sample. Conversely, color determinations are subject to errors caused by turbidity and by the fact that the wave length of the color in the sample may vary widely. Instruments of this type currently available do not satisfactorily compensate for these interferences and the data obtained from them does not correlate well with standard methods values.



B Colorimetric Analyzers

A second type of photometric instrument is designed to reproduce laboratory colorimetric procedures. That is, reagents are added to the sample to produce a color change proportional to the concentration of the material being determined. Since the sample is flowing continuously the reagents must be metered accurately and mixed thoroughly before photometric measurement. In a properly designed system, almost any colorimetric procedure can be duplicated and therefore the potential range of determinations is much wider than in the electrometric instruments.

A modification of the colorimetric instrument is the continuous titrator. In this system an indicator is added to the flowing sample and the reagent is added at a variable rate to maintain a constant color. The amount of reagent required is proportional to the concentration of the reactant in the sample, and the current used by the metering pump acts as a signal for the analyzer.

In spite of the apparent advantages of the photometric systems, certain special problems are inherent. The color and turbidity of the sample may interfere, the accumulation of slime in the cells may seriously reduce the sensitivity, and the limitations of the filter photometer (wide band pass) must be considered. Further, in most colorimetric procedures the amount of reagent required is proportional to the concentration range of the sample, a factor which would limit the applicability of the instrument.

C Parameters Measured Photometrically

Continuous analytical procedures have been developed for:

Turbidity
Color
Hardness
Residual Chlorine
Alkalinity
Fluoride
Silica
Phosphate
Phenols
Chemical Oxygen Demand

IV PERFORMANCE

To illustrate the quality of the data which may be obtained from an integrated water quality monitor, the following table shows the results of a performance test conducted on a prototype instrument supplied by one manufacturer. The "Acceptable" tolerances were selected as representative of the usual requirements for continuous data acquisition and may be too high or too low, depending upon the accuracy deemed necessary.

V CALIBRATION AND MAINTENANCE

A Calibration

In the parlance of the instrument manufacturer, calibration involves two steps: (1) the setting of the readout to zero value in the absence of sensor signal, and (2) setting of the readout to some standard value, such as 5mv. when the sensor signal is replaced by a standard signal. It is readily apparent that a calibration of this type adjusts the meter and amplifier to reproduce correctly the signal received from the sensing elements. It does not, however, "calibrate" the total instrument in terms of the concentration of measured substance in the sample.

For proper calibration, it is essential that the final readout of the instrument be adjusted to correspond to a true value for the measured material. Thus the only adequate calibration must involve the measurement of a standard solution, such as a buffer of known pH or a salt solution of known conductance. Since instrument systems may lack linear response to a wide signal range, the instrument should be calibrated at several points over the range of values anticipated. In some cases current instrument design has not included adequate means of replacing the sample with standard solutions for calibration purposes..

The frequency of recalibration depends upon both the stability of the analyzer and the sensor. In general, analyzer-amplifiers are more stable than the sensing systems now in use. The Basic Data Program,

PERFORMANCE DATA

Test	Acceptable deviation	Mean deviation found	% Acceptable
pH	± 0.1 unit	0.1 unit	80%
Temperature	± 1°	0.2°	85%
Conductance	± 5%	17 μmhos	99%
Dissolved Oxygen	± 0.5 mg/l	0.3 mg/l	73%

FWPCA, in performance specifications for monitoring instruments, has established a two-week period for unattended performance. While such instrument stability is desirable, circumstances of site location and sample characteristics, often necessitate more frequent checking and recalibration.

B Maintenance

It is unwise to assume that any stream monitoring instrument, no matter how well designed and built, can function adequately for extended periods of time without maintenance. Sensing elements are subject to physical, chemical, and biological actions, analyzer components may fail or perform erratically, and recorders may stop or fail to print. The frequency of maintenance depends upon a large number of factors both controllable and accidental and can be determined only by long-term testing and actual field experience. Ease of maintenance can be designed into the instrument by the use of replaceable electronic components and accessibility for cleaning of flow cells and sample lines.

The availability of trained personnel for checking and maintaining the instruments in the field is a key factor in the successful stream monitoring program. Such personnel need not be either electronic specialists or experienced analysts but they should have a working knowledge of the instrument system and a familiarity with the chemical principles involved in the use of standard solutions for calibration.

VI APPLICATIONS

Stream monitoring systems are now in use in a variety of locations throughout the United States. In most cases the instruments measure a single parameter or condition of the stream rather than a number of parameters. Some of the instruments employ well established measurement systems, for example pH and conductance, while others, are still largely experimental.

Because of the need for continuous stream data, the intensive interest on the part of regulatory agencies, and the development programs by instrument makers, it is safe to assume that the use of water quality monitoring instruments will increase significantly in the near future.

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Descriptors: Analytical Techniques, Instrumentation, Testing Monitoring, Control Systems, Data Collections, Data Transmission, Remote Sensing

ORGANIC ANALYSES IN POLLUTION SURVEYS

I INTRODUCTION

A Significance

Organic substances in surface water are present due to runoff, the metabolic products of microorganisms in streams, domestic wastes, and industrial wastes. Organic pollutants are significant because of their possible oxygen demand, color, and taste and odor potential. In addition, certain compounds may be persistent in the stream or exhibit chronic toxicity.

B Measurement

1 BOD, COD and carbon analysis have been used for general estimates of organic pollution. Results of each one carries a different connotation in terms of oxygen demand criteria:

a The BOD result includes an estimate of oxygen demand of biologically available materials including carbon, hydrogen, nitrogen, and sulfur components under favorable conditions.

b The COD includes an estimate of chemically oxidizable carbon hydrogen and sulfur components. It is useful to estimate first stage oxygen demand but the oxygen equivalent of unoxidized nitrogen must be added to estimate the long term demand.

c Total carbon (TC) or total organic carbon (TOC) reflect the carbon content specifically. It is possible to estimate oxygen equivalents on the basis of background information on sample characteristics.

The three criteria each reflect different approaches and sample characteristics. They may be used interchangeably in certain situations where relationships have been established. More importantly, they may be used to classify a sample on the basis of the various determinations. Recent developments include an instrumental methodology of estimating oxygen demand including the four major contributing components - carbon, hydrogen, nitrogen and sulfur.

2 The more specific analysis of organic contaminants in water is made difficult by the large number of complex organic substances in the sample as well as the very minute concentrations usually found. As a result, more extensive evaluation or organic pollution requires concentration and clean-up techniques followed by confirmatory identification.

II CONCENTRATION TECHNIQUES

A Carbon Filter

1 Procedure

The use of activated carbon for the adsorption of organic compounds is well known. The technique has been applied to the recovery and concentration of organics from water by means of the carbon filter. The filter consists of a glass cylinder 18 inches long and 3 inches in diameter packed with activated carbon. In use the water to be sampled is pumped through the filter until approximately 5000 gallons has been sampled. The carbon is then removed from the cylinder, dried at low temperature, and extracted serially with chloroform and ethyl alcohol. The organic compounds which have been recovered from the stream are then concentrated to small volume by evaporation of the solvent. By weighing the residue a value for the total chloroform extractables can be calculated. In most relatively clean streams the total organics recovered by this technique average less than 200 mg/l (200 ppb). Where industrial pollution is present, however, values as high as 1000 mg/l have been found.

2 Limitations

It is difficult to obtain quantitative information using the carbon filter because its performance is affected by such variables as flow rate, pH, particle size of carbon, quantity of particulate matter present, and type of organic compound being adsorbed. Recent research has shown that the system is most efficient when the flow rate is very slow and the total volume passed through the filter is very small. Thus the Water Pollution Surveillance System is now using modified units which pass 1000 liters (250 gallons) at a flow rate of 100 ml/min. (1/32 gpm).

B Liquid-Liquid Extraction

Liquid-liquid extraction techniques are based on the principle that an organic substance will distribute itself in some ratio between water and an organic solvent. If the ratio favors the organic solvent, that solvent can be used to extract the organic substance from water. The technique has limitations in water analysis, however, because the extraction of very large samples with very large amounts of solvent is required in order to recover large enough quantities of organics.

III CLEAN-UP PROCEDURES

A Solubility Separation

1 The residue from the carbon filter represents a mixture of most of the organic compounds present in the original stream sample. In order to obtain more information as to the nature of the organic contamination, it is necessary to separate the mixture into structural groups. This separation is accomplished by taking advantage of the differences in solubility of the groups in various aqueous solutions. The residue is first dissolved in ether and the undissolved material, called "ether insolubles" is removed by filtration. Then the ether solution of the organics is successively extracted with water, dilute HCl, dilute $NaHCO_3$, abd dilute NaOH, to yield the water soluble, amine, strong acid, weak acid, and neutral fractions of the original material. The weight of these fractions gives the relative proportions of these organic groups in the stream.

2 Through a knowledge of the relative amounts of various fractions present in the sample, the probable source of the organic contamination can be deducted. For example, a large portion of water soluble compounds might indicate natural materials from vegetation; a large amount of bases would indicate pyridine or similar nitrogen compounds.

B Chromatographic Separation

1 Using column chromatography with silica gel the neutral fraction can further be separated. Iso-octane elutes aliphatics; benzene elutes aromatics; finally, a 50:50 mixture of chloroform and methanol elutes the oxygenated fraction.

2 A high ratio of aliphatics to total extract would point to probable contamination by refinery wastes. Aromatic fractions are often odorous; such compounds as phenyl ether, ortho-nitrochlorobenzene and chlorinated hydrocarbon insecticides have been isolated from this fraction.

C Other Techniques

1 In recent years the application of thin layer and paper chromatographic techniques to the clean-up of the aromatic fraction has resulted in further separation and eventual identification of organic pollutants.

2 Employment of chemical modifications to form derivatives of selected compounds in a mixture may be employed

to improve specificity and sensitivity of infrared, gas chromatography or other determinative methods.

IV IDENTIFICATION

A Infrared spectra of cleaned-up extracts provide qualitative information as to the chemical nature of the compounds in the sample. In some cases the spectra have identified pure compounds.

B Gas chromatography applied to extracts also provides qualitative information. However, its greatest asset is the ability of certain detectors to provide quantitative information in the nanogram range. The technique is also used at times as a preliminary clean-up procedure before infrared identification.

V ANALYSIS OF SPECIFIC ORGANIC COMPOUNDS

A Phenols

1 Because of the importance of phenolic compounds as organic pollutants, analysis of wastes and stream samples for phenols is often necessary.

2 When interfering substances such as sulfur or nitrogen compounds are present, the sample must be distilled to remove the phenols from the interferences. Phenol concentrations above 0.1 mg/l, as in waste samples, may be determined by the aqueous 4-amino antipyrine method. For concentrations below 100 μg/l the 4AAP method is modified by a chloroform extraction of the colored complex. Sensitivity of the modified procedure is approximately 2 μg/l (2 ppb).

3 The 4AAP reagent produces color with a wide range of phenolic compounds, but the sensitivity varies with different phenols. Studies have shown that phenol itself produces as much or more color than other phenolic compounds usually encountered. Thus when phenol is used as a standard and the results are reported as phenol, the reported values represent the minimum amount of phenolic materials present in the sample.

B Surfactants

1 Surfactants include LAS (linear alkylate sulfonate) and alkyl sulfates (anionic) and ethoxy ether alkylates (non-ionics) among the active ingredients of synthetic detergents. The anionics and some of the non-ionics are determinable in sewage and river water by the formation of a stable colored complex with methylene blue. The method is accurate to approximately ± 0.1 mg/l river water samples.

2 Stress on use of biodegradable detergents has required adoption of methods to evaluate biodegradability. Improved tests are essential to estimate surfactants not included in the methylene blue active surfactants (MBAS) test.

C Taste and Odor

Certain organic pollutants can be measured according to their taste and odor potentials. An evaluation of the threshold odor number can be obtained by setting up a series of dilutions of sample. The dilution ratio at which odor is just detectable by a representative panel is defined as the threshold odor number.

D Oils and Grease

The presence of heavy oils and grease in surface water are indicative of industrial pollution. Methods for the general evaluation of such pollution consist of liquid extraction techniques. For a more specific analysis it is necessary to utilize chromatographic separation and infrared analysis.

E Pesticides

The FWPCA has published official methods for analysis of chlorinated hydrocarbons (3) applicable for measurement of microgram and picogram (10^{-6} and 10^{-9}g) per liter quantities along the following lines.

1 Chlorinated hydrocarbons

a Recovery of pesticides from water using the carbon filter or liquid-liquid extraction.

b Separation and identification of extracts.

1) Solubility and chromatographic separation serves as a clean-up procedure. Pesticides are found in the aromatic fraction.

2) Thin layer chromatography serves both as a clean-up technique and as a means for confirmatory identification.

3) Gas chromatography (both electron capture and microcoulometric) can be used for identification and quantitative assay of pesticides.

4) Paper chromatography can also be used for confirmatory identification.

c Infrared identification

Absolute identifications of pesticides can be made using micro infrared spectroscopy. However, it is necessary to have recovered 1-10 micrograms of pesticide when using the Perkin Elmer Model 421 instrument with 6×beam condenser and a 1.5 mm K Br disc.

2 Organic phosphorus

It is possible to measure organic phosphorus insecticides using electron capture gas chromatography following liquid-liquid extraction. However, more specificity and sensitivity can be obtained using phosphorus detectors based on a thermionic principle.

VI SUMMARY

Techniques necessary for the evaluation of organic pollution are presented. Such evaluation requires time-consuming and expensive analyses.

ACKNOWLEDGEMENT

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Descriptors:
Analytical Techniques, Chemical Analysis, Water Analysis, Organic Compounds, Water Pollution, Water Quality, Phenols, Surfactants, Taste, Odor, Oil, Pesticides. Insecticides.

INTERPRETATION OF CHEMICAL DATA

I Analytical results are an "imperfect" estimate of some real value sought as a means to interpret, predict, or control behavior. In sanitary engineering, this refers to some aspect of the aquatic environment; or for the purposes of this outline, a chemical determination related to water renovation.

A Interpretation of analytical results may be meaningful or misleading depending on the interpreter's recognition of various factors.

1 Sample validity in representation of a situation, location, time, or occurrence is a prerequisite for reliance on subsequent operations.

2 The analytical result may include a direct or indirect estimate of the information sought. It may be relatively specific or include a composite of several related items. Interpretation requires a careful evaluation of the information obtained in relation to the use of it.

3 It is probable that several related analysis, chemical, biological, or physical, will be used for interpretation of behavior. All of these should be consistent in terms of the applicable conditions. If not, it is essential to seek an explanation of the divergence.

B Analytical data commonly are defined in operational terms. i.e. the term is defined in relationship to the methods used to obtain a number estimate. For example, it is not possible to distinguish precisely that which is in soluble or suspended form in a given sample. The estimate of the suspended particulates is made on the basis of some filter media such as a membrane filter, glass fiber filter, asbestos mat filter, paper or other. The pore sizes vary from 0.45 microns in a commonly available membrane filter to significantly larger sizes such as for paper filters. A given sample may have particulates ranging from atomic or ionic sized materials dispersed in a soluble form to agglomerates of colloidal or settleable size. The pore size of the filter used determines the fraction retained in or on the filter media classified as suspended solids.

1 Analysis based upon two different filter media, therefore, may give different results for the estimate of sample suspended solids. Interpretation of results would require recognition of operational differences used. Similarly a given colorimetric test may give a high or low estimate based upon control of interference, reagent proportions, type of reagents and time or measurement factors.

2 Conditions under which the analysis was made such as personnel experience, work schedule, facilities, and incentive all contribute to validity of the results. An inexperienced person working in the field is unlikely to obtain as good an estimate as the professional under more ideal conditions.

3 Care in sampling and interference control has a real effect upon analytical validity.

II INTERPRETATION OF RESULTS

The interpretor must ask himself whether the analytical results apply to the situation and to the desired objectives. Are the assembled data usable, and do they contain the needed items? Close coordination is essential among the sampling, field, laboratory and management personnel to insure that the results are productive and consistent. Preliminary surveys are intended to define the problem but situations may change. Early recognition of possible situation changes hopefully should be followed by adjustment of the program to develop a meaningful package. This means that interpretation of data should be started with the first day's results and continue throughout the survey to be sure that the engineering, biological, chemical and field or laboratory results are consistent and that the questions asked do not lead to a mass of unneeded information or to a gap in required information. Certain selected examples illustrate problems that may arise.

A An activated sludge operation was checked for phosphorus removal with the following results.

90% of the phosphorus was removed on the basis of the determination of influent and effluent P. 53% of removed phosphorus was found in excess activated sludge solids.

1 Casual interpretation presents no problems but what happened to the difference between 90 and 53 or 37% of the P involved

a Consideration of the analysis involved revealed that the tested samples were filtered before determinative processing; therefore, the result was not total P but filterable P. The influent sample is likely to contain a larger fraction of total P in filterable form than the effluent sample because of the tendency for P to associate with biological growth. This tends to increase the indicated percentage removal of P.

b The 53% P in the excess solids may have been in error because of the adsorbed P washed into the filtrate, as a result of incomplete digestion of organic P or as a result of sampling problems. More complete digestion techniques were indicated to be necessary in this situation. Incomplete information is available for decision on washout and sampling. Total P on a whole sample basis preferable for material balance purposes.

c The information furnished is insufficient because total P was not included in the results. The fraction of P removed by filtration and that not included in solids digestion limits interpretation.

B A creek with about 0.7 cfs flow received the discharge of an ammonia plant stabilization pond discharging about 1 cfs. Less than 25 lbs. of nitrogen/day passed a given point above the lagoon discharge. Lagoon discharge was about 7200 lbs./day of nitrogen (N) as ammonia. Within 3 miles of flow the stream contained less than 800 lbs. N in the daily flow. Organic N varied but not enough to account for 90% N disappearance. Oxidized N was negligible in the sample data. Further, there were occasional increases of NH_3-N in the flow that could not be attributed to tributaries or shoreline occupants.

1 Apparent lack of conversion of ammonia to oxidized form is partially explained in terms of the hydrology involved. The stream was a series of pools and riffles. Substantial quanitites of ammonia may have been converted to nitrates by attached growth in the riffles but converted to N gas by denitrification in the pools where little, if any, DO was detected even during daylight. Under these conditions little oxidized N would be found regardless of rapid oxidation in the riffles because it's hard to sample riffled flow.

2 Volatility of the N as NH_3 from the flow would be a possibility considering 7000 plus lbs. of NH_3-N in 1.7 cfs or about 1.1 mgd of flow. 7000 in 9,200,000 represents about 750 mg/l as NH_3-N. Volatility in the riffle areas could account for reduction of N to the vicinity of 200 mg/l but it must be recognized that the flow used was that below the lagoon and did not include downstream dilution of more than 5 times within 2 miles of flow. It does not appear likely that volatility could account for more than 2/3 of the loss in NH_3-N but may have been larger or smaller.

3 Unexplained rises in NH_3-N are likely to be the result of deamination in pooled areas. Solids discharged would settle in pools, become anaerobic with organic N hydrolyzing to amines and subsequently converted to acids and ammonia.

C A Survey of the Upper Missouri River

Total carbon and total organic carbon (TC and TOC) determinations indicated that there was a general tendency for gradual increases from upstream to downstream sampling points above Kansas City, Missouri. Certain increases were not accountable in relation to customary interpretation.

1 This illustrates a problem related to the use of a relatively new analytical criterion for which a history of characteristics to guide interpretation is scant.

2 Conventional oxygen demand criteria tend to show a sharp rise below a source of pollution followed by a decrease with downstream travel to the next source of pollution. Carbon data apparently need additional or different correlation considerations.

3 A gradual increase in alkalinity may be expected with downstream travel, effects of deposition and resuspension may be expected, the conversion of oxygen demanding materials into humis may be expected. Experience under a variety of situations is required to reveal other expectancies needed for interpretation of these data.

III The examples illustrate three different situations leading to some loss of clarity: i.e., an analytical yield problem, a situation in which the usual questions didn't quite fit, and one in which a new criterion required a different approach.

It would be desirable to be in a position to "follow through" and clarify each situation. Survey economics, manpower and time rarely permit an unlimited application of effort. Judicious use of past experience and knowledge of the situation are useful to direct resources into productive channels; prompt review of collected data for apparent trends helps to keep them on path or adjust accordingly.

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Descriptors:
Analytical Techniques, Water Analysis, Chemical Analysis, Data Interpretation, Basic Data Collections

BIOCHEMICAL OXYGEN DEMAND TEST PROCEDURES

I OXYGEN DEMAND OF POLLUTED WATERS

Established practice includes common use of the BOD test as a tool for estimation of the bio-oxidizable fraction of surface waters or wastewaters discharged to them. Any index including a quantity per unit time such as the BOD_5 is a rate expression. The ultimate demand is more important than any one point on the progression. The results of a bottle test with minimum seeding and quiescent storage are not likely to be as high as those on the same influent in a mixing situation and abundant seed of secondary treatment or receiving waters. The BOD_5 is "a" fraction of total oxygen requirements.

A The particular technique used for BOD commonly is specified by State agencies and/or supervisors. They are required to interpret the results as obtained by laboratory testing. It is essential that the tester and the interpreters have a common understanding of what was done and how. It is highly advisable to maintain a given routine until all concerned agree upon a change.

1 Each particular routine has many undefinable factors. The particular routine is not as important as the consistency and capability with which the result was obtained.

2 This outline and Standard Methods$^{(1)}$ discusses several valid approaches for obtaining BOD results. Selection of "method" is not intended in this outline or in the EPA Methods Manual$^{(2)}$.

B The common 5-day incubation period for BOD testing is a result of tradition and cost. Initial lags are likely to be over and some unknown fraction of the total oxidizable mass has been satisfied after 5 days.

C A series of observations over a period of time makes it possible to estimate the total oxidizable mass and the fraction oxidized or remaining to be oxidized at any given time. The problem is to define the shape of the deoxygenation pattern and its limits. A fair estimate of the shape of the deoxygenation pattern is available by observations at 1, 2, or 3 days, 7 days and 14 days. Increased observations are desirable for more valid estimates of curve shape, rate of oxidation and total oxidizable mass or ultimate BOD.

D Increasing impoundment of surface waters and concurrent increases in complexity and stability of wastewater components emphasize the importance of long-term observation of BOD. The 5-day observation includes most of the readily oxidizable materials but a very small fraction of the stable components that are the main factors in impoundment behavior.

II DIRECT METHOD

A With relatively clean surface waters, the BOD may be determined by incubation of the undiluted sample for the prescribed time interval. This method is applicable only to those waters whose BOD is less than 8 mg/l and assumes the sample contains suitable organisms and accessory nutrients for optimum biological stabilization.

B Treated effluents, polluted surface waters, household and industrial wastewaters commonly require dilution to provide the excess oxygen required for the oxygen demand determination. General guidelines for dilution requirements for a given BOD range in terms of the percent of sample in BOD dilution water are:

For a 5-day BOD of
5-20 mg/l, use 25 to 100% sample

For a BOD of
20-100 mg/l, use 5 to 25% sample

For a BOD of
100-500 mg/l, use 1 to 5% sample

For a BOD of
500-5000 mg/l, use 0.1 to 1.0% sample

III PROCEDURES

A Cylinder Dilution Technique

1 Using an assumed or estimated BOD value as a guide, calculate the factors for a range of dilutions to cover the desired depletions. Those dilutions ranging from a depletion of 2 mg/1 and a residual of 1 mg/1 are most reliable. At least three dilutions in duplicate should be used for an unknown sample.

2 Into a one-liter graduate cylinder (or larger container if necessary) measure accurately the required amount of mixed sample to give one liter of diluted waste. Fill to the one liter mark with dilution water. Carefully mix. The initial DO by calculation includes IDOD (VIII) a determined initial does not. Both are essential to estimate significance of IDOD. Entrapment of air bubbles during manipulation must be avoided.

3 Siphon the mixture from the cylinder into three 300 ml glass stoppered bottles, filling the bottles to overflowing.

4 Determine the DO concentration on one of the bottles by the appropriate Winkler modification and record as "Initial DO".

5 Incubate the two remaining bottles at 20°C in complete darkness. The incubated bottles should be water-sealed by immersion in a tray or by using a special water-seal bottle.

6 After 5 days of incubation, or other desired interval, determine the DO on the bottles. Average the DO concentration of the duplicates and report as "Final DO".

B Direct Dilution Technique

1 It may be more convenient to make the dilution directly in sample bottles of known capacity. A measured volume of sample may be added (as indicated in A-1) above, and the bottle filled with dilution water to make the desired sample concentration for incubation. In this case, the sample must be precisely measured, the bottle carefully filled, but not overfilled, and the bottle volumes comparable and known. Precision is likely to be poorer than for cylinder dilution.

2 Continue the procedure as in A-4, 5, and 6 above.

C Seeded Cylinder Dilution Technique

1 Many wastewaters may be partially or completely sterile as a result of chlorination, effects of other toxic chemicals, heat, unfavorable pH or other factors detrimental to biological activity. Validity of the BOD result depends upon the presence of organisms capable of prompt and effective biodegradation and favorable conditions during the particular test. Correction of the cause resulting in sterilization must be corrected by adjustment, dilution, etc., prior to reinoculation to achieve meaningful BOD data. Receiving water, biologically treated effluents, and soil suspensions are a good source of organisms likely to be adapted for stabilization of wastewaters. Untreated wastewaters provide numerous organisms but are likely to contain nutrients contributing to excessive seed corrections and may require appreciable time for adaptation before test waste oxidation becomes significant.

2 The amount of added inoculant must be determined by trial. The concentration added should initiate biochemical activity promptly but should not exert enough oxygen demand to unduly reduce the oxygen available for sample requirements.

3 Estimate the sample concentration desired in accordance with A-1 and C-2 above and add the sample aliquot to the dilution cylinder.

4 Add approximately half of the required amount of dilution water to the sample and mix. This is necessary to assure that the concentrated waste does not exert a toxic effect on the seed organisms.

5 Measure a suitable aliquot of seed into the bottle or cylinder and fill with dilution water. Mix the combined sample, seed and dilution water without excessive air entrainment.

6 Continue as in III-A steps 4, 5, and 6 above.

IV INTERPRETATION OF RESULTS

Standard Methods(1) includes a calculation section that is valid and concise. Preceding it are details of reagent preparation and procedures for the test. These will not be reprinted here. This section considers certain items that may cause concern about the validity of results unless they are carefully considered and controlled.

A The initial DO of the BOD test obviously should be high. The method of attaining a high DO can trap the analyst.

1 Aeration of dilution water is the most commonly considered treatment. This technique does produce a high DO but it is a treacherous ally.

a Dirty air passing through clean dilution water can produce clean air and dirty water. This is a simple air-washing operation. Filtering the entering air stream may remove brickbats and 2 x 4's, but filters tend to pass organic gases, fine aerosols, and particulates.

b A stream of air passing through water tends to cool the water by evaporation 1 to 3°C below ambient temperature. The cooled liquid picks up more DO than it can hold at ambient temperature. The physical loss of oxygen may produce an erroneously high depletion value for a determined initial DO, or a low depletion on a calculated initial DO. Erroneous blanks are a particular concern. The dilution water temperature/DO shift is critical.

2 Raising DO by allowing the sample to equilibrate in a cotton-plugged bottle for 2 or 3 days permits oxygenation with minimum air volume contact.

3 Shaking a partially filled bottle for a few seconds also oxygenates with minimum opportunity of gas washing contamination, supersaturation, or temperature changes.

B Seeding always is a precarious procedure but a very necessary one at times. Often the application of seed corrections is a "________if you do, _______if you don't" situation. Hopefully, seed corrections are small because each individual biological situation is a "universe" of its own.

1 Unstable seeding materials such as fresh wastewater have "seed" organisms characteristic of their origin and history. Saprophytes resulting in surface water stabilization may be a small fraction of the population. Reactable oxygen-demanding components produce excessive demands upon test oxygen resources.

2 A seed containing viable organisms at a lower energy state because of limited nutritional availability theoretically is the best available seed source. An organism population grown under similar conditions should be most effective for initiating biochemical activity as soon as the nutrient situation favors more activity. The population should not be stored too long because organism redistribution and die-out become limiting. This type of seed would most likely be found in a surface water or a treatment plant effluent with a history of receiving the particular material under consideration.

3 Seed sources and amounts can only be evaluated by trial. Different seed sources and locations require checkout to determine the best available material from a standpoint of rapid initiation of activity, low correction, and predictable high oxygen depletion under test.

C Chlorination and BOD results fundamentally are incompatible. Chlorination objectives include disinfection as the number one goal. Chlorine is notoriously non-specific in organism effects. Chlorine acts like an oxidant in the DO determination. Test organisms are less suitable for activity than they were before chlorination. Nutrients may be less available after chlorination. Certainly the conditions are less suitable for biological response after chlorination. Dechlorination is feasible with respect to the oxidizing power of chlorine, but many organic chlorine compounds that do not show strong oxidizing action still have toxic effects on biologic response.

Numbers are obtainable after dechlorination and reseeding. The meaning of these numbers is obscure. At least two states (New York and New Jersey) specify BOD's before chlorination only.

V PRECISION OF THE BOD TEST

A The DO test precision often has been used to suggest precision of the BOD result.

DO precision is a relatively minor and controllable factor contributing to BOD results. Other factors such as organism suitability, members, adaptation and conditional variables are much more difficult to control or to evaluate.

B The Analytical Reference Service report on Water Oxygen Demand, July, 1960 (Sample type VII) included the results of seeded samples of glucose-glutamic acid BOD results from 34 agencies on 2, 3, 5 and 7 day incubations.

The relative geometric standard deviation (average) was 19% on 2% sample and 24% on 1% sample concentrations. Rate coefficients ranged from 0.10 to 0.27 with a median of 0.16 from 21 different laboratories that participated in rate studies.

VI ALTERNATE BOD TECHNIQUES

Reaeration methods are becoming increasingly popular in order to approach more nearly the actual waste concentration in the receiving water. It is common to obtain "sliding" BOD results related to the concentration of waste in a series of dilutions of the same sample. This may result from greater possibilities for toxic effects at higher concentrations, or to a different selection of organisms and change in oxidation characteristics at low concentrations of sample. The most reliable estimate of stream behavior is likely to be from that dilution closest to the wastewater dilution in the receiving water.

A Reaeration can be accomplished by the usual series techniques by dumping all of the remaining sealed bottles into a common container when the residual DO reaches about 1.0 mg/1. After reoxygenation, the remaining bottles are refilled and a new initial DO determined. Subsequent dissolved oxygen depletions are added incrementally as a summation of the total oxygen depletion from the start of the test. If necessary, the reaeration technique may be performed several times but at a sacrifice of double DO determinations for each day on which reaeration occurs.

B Special methods of reaeration have evolved to minimize the extra manipulation for reaeration of individual sample dilutions.

1 Elmore Method

A relatively large volume of the sample is stored in an unsealed bottle. Small bottles are withdrawn in sets of 5 or more, sealed, incubated, and the DO determined at appropriate intervals. When the DO concentration in the smaller bottles reaches 1.0 mg/1, a new set is withdrawn from the large unsealed bottle, after reoxygenation if necessary.

2 Orford Method

The deoxygenation is carried out in a large sealed jug from which samples for DO are withdrawn at appropriate intervals. To maintain the waste level and a sufficient DO in the jug, additional waste is added from a second open container. See diagram.

C Excess oxygen may be provided by oxygenation with commercial oxygen instead of with air to increase the initial oxygen content for incubation while limiting the number of dilutions or reaeration steps. When oxygen is used in place of air the oxygen saturation in water at 20°C is about 40 mg/1 instead of 9 mg/1. Limited results are available hence the analyst must verify his technique. The DO tends to decrease as soon as the bottle is opened hence, about 35 mg/1 of oxygen content is the top of the practical working concentration. There has been no evidence that the biota is inhibited by the higher oxygen content with respect to BOD progression.

D Reaeration or Oxygenation Advantages and Limitations.

1 Reaeration expands the range of BOD results obtainable directly at field concentrations, but is not advisable for applications when the sample BOD exceeds 50 mg/1.

2 Dilution water problems are eliminated, to the extent that the stream sample may be tested without dilution.

3 Incubator storage space becomes a real problem for multiple sample routine.

VII Dissolved oxygen electrodes, polarographic and others, are feasible for use in BOD determinations, often making it possible to make an estimate of DO or BOD when sample interference prevents a valid Winkler DO determination.

Electronic probe DO makes it possible to determine many successive DO's at different time intervals on the same bottle with negligible sample loss. Reaeration or extended time series, therefore, are more feasible.

Another outline in this series describes response of reaerated BOD_5 with electronic DO probes.

A It is the responsibility of the analyst to evaluate:

1 Applicability of the specified technique and sample.

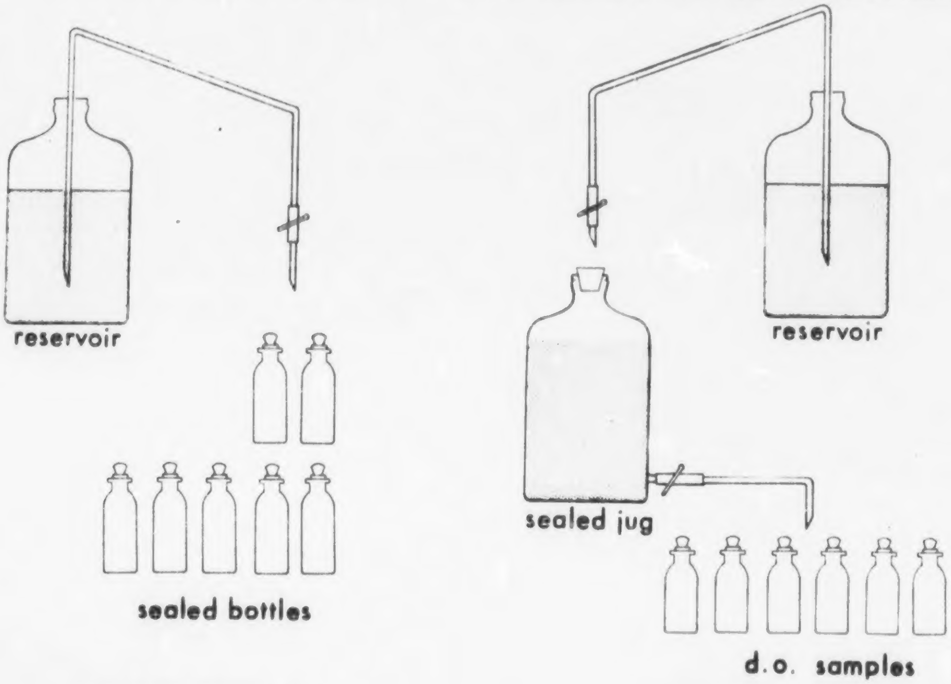
2 To determine requirements for mixing and possible thermal effects while mixing in terms of instrument response and biochemical reaction.

3 To evaluate long-term calibration or standardization and their effects upon precision and accuracy of the BOD result.

VIII IMMEDIATE DISSOLVED OXYGEN DEMAND (IDOD)

Immediate dissolved oxygen demand includes dissolved oxygen utilization requirements of substances such as ferrous iron, sulfite and sulfide which are susceptible to high rate chemical oxidation.

REAERATION METHODS FOR B.O.D. DETERMINATION



ELMORE METHOD

ORFORD METHOD

A The IDOD is an apparent response as indicated by a specified technique. Since DO titration is based upon iodine titration, any factor that causes I_2 response different from that produced by the reaction of KI and molecular oxygen confuses the IDOD determination.

B IDOD Determination

1 The IDOD determination includes the determination of DO on a sample and dilution water separately. A waste likely to have a significant IDOD is unlikely to show a DO.

2 According to mixing theory, it should be possible to calculate the DO of any definite mixture of the sample and dilution water from the DO of component parts and their proportion.

3 The same relative proportions of sample and dilution water should be mixed without air entrainment and the DO determined after the arbitrarily selected time of 15 minutes.

4 Any difference between the calculated initial DO as obtained in 2 above, and the DO determined in 3 above, may be designated as IDOD.

5 Sample aeration, DO interference, and other factors affect results for IDOD.

C Sample Calculation of IDOD

1 Sample DO checked and shown to be 0.0 mg/l

Dilution water DO found to be 8.2 mg/l

Assume a mixture of 9 parts of dilution water and 1 part (V/V) of sample.

Calculated DO =

$1 \times 0 = 0$

$9 \times 8.2 = 73.8$

10 parts of the mixture contain 73.8/10 or 7.4 mg DO/l. Note that mixing has reduced the DO concentration because the original amount is present in a larger package.

2 The mixture described above was held for 15 minutes and the DO determined was 4.3 mg/l.

$$\text{IDOD} = \text{DO}_{\text{calc}} - \text{DO}_{\text{detm}} \times \frac{100}{\%\ \text{sample used}}$$

$$= 7.4 - 4.3 \times 10$$

$$= 31 \text{ mg IDOD/l}$$

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This outline was prepared by F. J. Ludzack, Chemist, National Training Center, EPA, WPO, Cincinnati, OH 45268.

Descriptors: Biochemical Oxygen Demand, Chemical Analysis, Chemical Oxygen Demand, Dissolved Oxygen, Water Analysis

LABORATORY FOR BIOCHEMICAL OXYGEN DEMAND TEST

I REAGENTS

A Winkler Dissolved Oxygen (Alsterberg Modification)

1 Manganous sulfate

2 Alkaline iodide-azide

3 Sulfuric acid (concentrated)

4 0.037N sodium thiosulfate

5 Starch solution

B BOD Test

Standard Methods Dilution Water

II EQUIPMENT

A DO Bottles (300 ml)

Three bottles for each dilution (9 bottles for each test); one bottle for initial DO and 2 bottles for incubation, each dilution.

B Siphon

C Aspirator Bulb

D 1-liter Graduated Cylinder

E Aerator Plunger

F 3 - 2 ml Transfer Pipettes

G 1 100-ml Graduated Cylinder
1 25-ml Graduated Cylinder

H 2 500-ml Erlenmeyer Flasks

I 25-ml or 5--ml Burette, stand

J BOD Incubator (20°C)

III PROCEDURE

A Preparation of Dilution

1 Mix sample thoroughly

2 Prepare separately 1%, 5%, and 10% dilutions of the sample: Add 10 ml, 50 ml, and 100 ml of sample, respectively, to the 1-liter graduated cylinder, and fill to the 1-liter mark with BOD dilution water mix.

3 Siphon contents of graduated cylinder into 3 DO bottles. Repeat for each dilution (1%, 5% and 10%).

B Winkler DO Determination (on one of the three bottles for each dilution) maintain a full siphon tube throughout transfer.

1 Add 2 ml of manganous sulfate solution, introduced below the liquid surface.

2 Add 2 ml of alkaline iodide-azide solution, introduced below the liquid surface, and immediately stopper the bottle.

3 Mix the contents of the bottle by inverting 10-15 times.

4 Permit floc to settle halfway down the bottle, and again invert the bottle and mix the contents.

5 After settling, add 2 ml of sulfuric acid (concentrated), stopper, and mix.

6 Pour entire contents of the bottle into a 500 ml Erlenmeyer flask.

7 Titrate with 0.037N sodium thiosulfate solution to a pale straw color; then add 5 drops of starch solution, and continue titration until the blue color disappears.

8 Record the volume of sodium thiosulfate used; the volume in ml represents the

number of milligrams of dissolved oxygen per liter of sample.

9 Incubate the other two bottles for each sample dilution, inverted under water or with stopper-well filled, 5 days at 20° C in the dark.

10 Remove bottles after incubation period and perform Winkler DO determinations as described in steps 1 thru 8.

IV CALCULATIONS

$$\text{BOD} = \frac{(\text{initial DO} - \text{final DO}) \times 100}{\text{\% of sample in dilution incubated}}$$

Example:

If the DO initial was 8.0

DO final was 3.2

2% sample tested

then:

$$\text{BOD} = \frac{(8.0 - 3.2) \times 100}{2}$$

$$= \frac{4.8 \times 100}{2}$$

$$= 4.8 \times 50$$

$$= 240.0 \text{ mg BOD/l}$$

B If the sample was suspected of having an immediate dissolved oxygen demand (IDOD), a calculated initial would be required. The calculated initial would be higher than the determined initial if an IDOD was involved.

Sample DO = 0.0 mg/l

Dilution Water DO = 8.2 mg/l

2×0.0 = 0.0 oxygen units

$$\frac{98 \times 8.2}{100 \text{ parts}} = \frac{804.\text{oxygen units}}{804 \text{ total oxygen units}}$$

$$\text{Unit DO concentration} = \frac{804}{100} = 8.04 \text{ mg/l}$$

for the calculated DO_1, since the DO_1 was 8.0, any IDOD must be assumed negligible.

This outline was revised by C. R. Feldmann, Chemist, EPA, WPO, National Training Center, Cincinnati, OH 45268.

Descriptors:
Biochemical oxygen demand, Chemical Analysis, Chemical Oxygen Demand, Dissolved Oxygen, Water Analysis

CHEMICAL OXYGEN DEMAND AND COD/BOD RELATIONSHIPS

I DEFINITION

A The Chemical Oxygen Demand (COD) test is a measure of the oxygen equivalent that portion of the organic matter in a sample that is susceptible to oxidation under specific conditions of oxidizing agent, temperature and time.

B A variety of terms have been and are used for the test described here as COD:

1 Oxygen absorbed (OA) primarily in British practice.

2 Oxygen consumed (OC) preferred by some, but unpopular.

3 Chemical oxygen demand (COD) current preference.

4 Complete oxygen demand (COD) misnomer.

5 Dichromate oxygen demand (DOC) earlier distinction of the current preference for COD by dichromate or a specified analysis such as Standard Methods.

6 Others have been and are being used. Since 1960, terms have been generally agreed upon within most professional groups as indicated in I-A and B-3 and the explanation in B-5.

C The concept of the COD is almost as old as the BOD. Many oxidants and variations in procedure have been proposed, but none have been completely satisfactory.

1 Ceric sulfate has been investigated, but in general it is not a strong oxidant.

2 Potassium permanganate was one of the earliest oxidants proposed and until recently appeared in Standard Methods (9th ed.) as a standard procedure. It is currently used in British practice as a 4-hr. test at room temperature.

a The results obtained with permanganate were dependent upon concentration of reagent, time of oxidation, temperature, etc., so that results were not reproducible.

3 Potassium iodate or iodic acid is an excellent oxidant but methods employing this reaction are time-consuming and require a very close control.

4 A number of investigators have used potassium dichromate under a variety of conditions. The method proposed by Moore at SEC is the basis of the standard procedure.(1, 2) Statistical comparisons with other methods are described. (3)

5 Effective determination of elemental carbon in wastewater was sought by Buswell as a water quality criteria.

a Van Slyke(4) described a carbon determination based on anhydrous samples and mixed oxidizing agents including sulfuric, chromic, iodic and phosphoric acids to obtain a yield comparable to the theoretical on a wide spectrum of components.

b Van Hall, et al.,(5) used a heated combustion tube with infrared detection to determine carbon quickly and effectively by wet sample injection.

6 Current development shows a trend to instrumental methods automating

conventional procedures or to seek elemental or more specific group determination.

II RELATIONSHIP OF THE COD TEST WITH OTHER OXIDATION CRITERIA IS INDICATED IN TABLE 1.

A Table 1

Test	Test Temp. °C	Reaction time	Oxidation system	Variables
BOD	20	days	Biol. prod. Enz. Oxidn.	Compound, environment, biota, time, numbers. Metabolic acceptability, etc.
COD	145	2 hrs.	50% H_2SO_4 $K_2Cr_2O_7$ May be catalyzed	Susceptibility of the test sample to the specified oxidation
IDOD	20	15'	Diss. oxyg.	Includes materials rapidly oxidized by direct action, Fe^{+2}, SH.
Van Slyke Carbon detn.	400+	1 hr.	H_3PO_4 HIO_3 H_2SO_4 $K_2Cr_2O_7$ Anhydrous	Excellent approach to theoretical oxidation for most compounds (N-nil)
Carbon by combustion +IR	950	minutes	Oxygen atm. catalyzed	Comparable to theoretical for carbon only.
Chlorine Demand	20	20 min.	HOCl soln.	Good NH_3 oxidn. Variable for other compounds.

B From Table 1 it is apparent that oxidation is the only common item of this series of separate tests.

1 Any relationships among COD & BOD or any other tests are fortuitous because the conditions of test tend to give results indicating the susceptibility of a given sample to oxidation under specified conditions that are different for each test.

2 If the sample is primarily composed of compounds that are oxidized by both procedures (BOD and COD) a relationship may be established.

a The COD procedure may be substituted (with proper qualifications) for BOD or the COD may be used as an indication of the dilution required for setting up BOD analysis.

b If the sample is characterized by a predominance of material that can be chemically, but not biochemically oxidized, the COD will be greater than the BOD. Textile wastes, paper mill wastes, and other wastes containing high concentrations of cellulose have a high COD, low BOD.

c If the situation in item b is reversed the BOD will be higher than the COD. Distillery wastes or refinery wastes may have a high BOD, low COD, unless catalyzed by silver sulfate.

d Any relationship established as in 2a will change in response to sample history and environment. The BOD tends to decrease more rapidly than the COD. Biological cell mass or detritus produced by biological action has a low BOD but a relatively high COD. The COD/BOD ratio tends to increase with time, treatment, or conditions favoring stabilization.

III ADVANTAGES AND LIMITATIONS OF THE COD TEST(2) AS RELATED TO BOD

A Advantages

1 Time, manipulation, and equipment costs are lower for the COD test.

2 COD oxidation conditions are effective for a wider spectrum of chemical compounds.

3 COD test conditions can be standardized more readily to give more precise results.

4 COD results are available while the waste is in the plant, not several days later, hence, plant control is facilitated.

5 COD results are useful to indicate downstream damage potential in the form of sludge deposition.

6 The COD result plus the oxygen equivalent for ammonia and organic nitrogen is a good estimate of the ultimate BOD for many municipal wastewaters.

B Limitations

1 Results are not applicable for estimating BOD except as a result of experimental evidence by both methods on a given sample type.

2 Certain compounds are not susceptible to oxidation under COD conditions or are too volatile to remain in the oxidation flask long enough to be oxidized.

Ammonia, aromatic hydrocarbons, saturated hydrocarbons, pyridine, and toluene are examples of materials with a low analytical response in the COD test.

3 Dichromate in hot 50% sulfuric acid requires close control to maintain safety during manipulation.

4 Oxidation of chloride to chlorine is not closely related to BOD but may affect COD results.

5 It is not advisable to expect precise COD results on saline water.

IV BACKGROUND OF THE STANDARD METHODS COD PROCEDURE

A The COD procedure(1) considered dichromate oxidation in 33 and 50 percent sulfuric acid. Results indicated preference of the 50 percent acid concentration for oxidation of sample components. This is the basis for the present standard procedure.

B Muers(6) suggested addition of silver sulfate to catalyze oxidation of certain low molecular weight aliphatic acids and alcohols. The catalyst also improves oxidation of most other organic components to some extent but does not make the COD test universally applicable for all chemical pollutants.

C The unmodified COD test result (A) includes oxidation of chloride to chlorine. Each mg of chloride will have a COD equivalent of 0.23 mg. Chlorides must be determined in the sample and the COD result corrected accordingly.

1 For example, if a sample shows 300 mg of COD per liter and 200 mg Cl^- per liter the corrected COD result will be 300 -(200 x 0.23)or 300 - 46 = 254 mg COD/l on a chloride corrected basis.

2 Silver sulfate addition as a catalyst tends to cause partial precipitation of silver chloride even in the hot acid solution. Chloride corrections are questionable unless the chloride is oxidized before addition of silver sulfate, i.e., reflux for 15 minutes for chloride oxidation, add Ag_2SO_4, and continue the reflux or use of $HgSO_4$(D).

D Dobbs and Williams (7) proposed prior complexation of chlorides with $HgSO_4$ to prevent chloride oxidation during the test. A ratio of about 10 of Hg^{++} to 1 of Cl^- (wt. basis) appears essential. The Cl^- must be complexed in acid solution before addition of dichromate and silver sulfate.

1 For unexplained reasons the $HgSO_4$ complexation does not completely prevent chloride oxidation in the presence of high chloride concentrations.

2 Factors have been developed to provide some estimate of error in the result due to incomplete control of chloride behavior. These tend to vary with the sample and technique employed.

E It is not likely that COD results will be precise for samples containing high chlorides. Sea water contains 18000 to 21000 mg Cl^-/l normally. Equivalent chloride correction for COD exceeds 4000 mg/l. The error in chloride determination may give negative COD results upon application of the correction.

Incomplete control of chloride oxidation with $HgSO_4$ may give equally confusing results.

$HgSO_4$ appears to give precise results for COD when chlorides do not exceed about 2000 mg/l. Interference increases with increasing chlorides at higher levels.

F The 12th edition of Standard Methods reduced the amount of sample and reagents to 40% of amounts utilized in previous editions. There has been no change in the relative proportions in the test. This step was taken to reduce the cost of providing expensive mercury and silver sulfates required. Results are comparable as long as the proportions are identical. Smaller aliquots of sample and reagents require more care during manipulation to promote precision.

G The EPA Methods for COD

1 For routine level COD (samples having an organic carbon concentration greater than 15 mg/liter and a chloride concentration less than 2000 mg/liter), the EPA specifies the procedures found in Standard Methods (2) and in ASTM(8).

2 For low level COD (samples with less than 15 mg/liter organic carbon and chloride concentration less than 2000 mg/liter), EPA provides an analytical procedure (9). The difference from the routine procedure primarily involves a greater sample volume and more dilute solutions of dichromate and ferrous ammonium sulfate.

3 For saline samples (chloride level exceeds 2000 mg/liter), EPA provides an analytical procedure(9) involving preparation of a standard curve of COD versus mg/liter chloride to correct the calculations. Volumes and concentrations for the sample and reagents are adjusted for this type of determination.

V The precision of the unmodified COD result shows a standard deviation of ± 4% of the mean (3) on low chloride samples. Silver sulfate modified COD results are likely to show a standard deviation about twice that without catalysis, due to questionable chloride behavior. The determination of chloride frequently shows a coefficient of variation ($s/\bar{x}$) of 10 to 15%, hence high chloride samples result in COD precision controlled more by chloride behavior than organic oxidation.

VI REMARKS PERTINENT TO EFFECTIVE COD DETERMINATIONS INCLUDE:

A Sample size and COD limits for 0.25 N reagents are approximately as given. For 0.025 N reagents multiply COD by 0.1. Use the weak reagent for COD's in the range of 5-50 mg/1, (low level).

Sample Size	mg COD/1
20 ml	2000
10 ml	4000
5 ml	8000

B Most organic materials oxidize relatively rapidly under COD test conditions. A significant fraction of oxidation occurs during the heating upon addition of acid. The color change of dichromate remaining. If the mixed sample color changes from yellow to green after acid addition the sample was too large. Discard without reflux and repeat with a smaller aliquot until the color after mixing does not go beyond a brownish hue. The dichromate color change is less rapid with sample components that are slowly oxidized under COD reaction conditions.

C Chloride concentrations should be known for all test samples and results interpreted accordingly.

D Special precautions advisable for the regular COD procdure and essential when using 0.025 N reagents include:

1 Keep the apparatus assembled when not in use.

2 Plug the condenser breather tube with glass wool to minimze dust entrance.

3 Wipe the upper part of the flask and lower part of the condenser with a wet towel before disassembly to minimize sample contamination.

4 Steam out the condenser after use for high concentration samples and periodically for regular samples. Use the regular blank reagent mix and heat, without use of condenser water, to clean the apparatus of residual oxidizable components.

5 Distilled water and sulfurnic acid must be of very high quality to maintain low blanks on the refluxed samples for the 0.025 N oxidant.

ACKNOWLEDGEMENT:

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This outline was prepared by F. J. Ludzack, Chemist, EPA, WPO, National Training Center, Cincinnati, OH 45268.

Descriptors: Biochemical Oxygen Demand, Chemicals, Chemical Analysis, Chemical Oxygen Demand, Chemical Reactions, Chlorides, Dissolved Oxygen, Organic Compounds, Oxidation, Oxygen, Oxygen Demand, Oxygen Requirements, Saline Water, Water Analysis

LABORATORY PROCEDURE FOR ROUTINE LEVEL CHEMICAL OXYGEN DEMAND

I REAGENTS

A Standard Potassium Dichromate (0.250 N):

Dissolve 12.259 g of primary standard grade $K_2Cr_2O_7$, which had been dried at 103° C for two hours, in distilled water and dilute to one liter.

B Ferrous Ammonium Sulfate (0.1N):

Dissolve 39 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water.
Carefully add 20 ml of concentrated H_2SO_4.
Cool and dilute to one liter.

C Ferroin Indicator:

Dissolve 1.485 g 1, 10-phenanthroline monohydrate and 0.695 g $FeSO_4 \cdot 7H_2O$ in water and dilute to 100 ml. This indicator may be purchased already prepared.

D Concentrated Sulfuric Acid (36 N):

E Mercuric Sulfate: Analytical Grade

F Silver Sulfate: Analytical Grade

G Concentrated Sulfuric Acid - Silver Sulfate:

Dissolve 22 g of silver sulfate in a 9 lb bottle of concentrated sulfuric acid. (1-2 days required for dissolution)

II EQUIPMENT PREPARATION

Before use, the Erlenmeyer flask (500 ml, 24/40 standard taper joint) and reflux condenser (Friedrichs, 24/40 standard taper joint) should be steamed out to remove trace organic contaminants. Add 10 ml of 0.250 N $K_2Cr_2O_7$, 50 ml of distilled water, and several bumping stones to the flask. Carefully add 20 ml of concentrated H_2SO_4 and mix thoroughly. Connect the flask and condenser, but do not turn on the water to the condenser. Boil the mixture so that steam emerges from the top of the condenser for several minutes. Cool the mixture, carefully discard the acid, and rinse the condenser and flask with distilled water. In order to prevent contamination from air-borne particles, the top of the condenser should be lightly plugged with glass wool during storage and use.

III STANDARDIZATION OF FERROUS AMMONIUM SULFATE

A Dilute 10.0 ml of the standard potassium dichromate to about 100 ml with distilled water.

B Add 30 ml of concentrated H_2SO_4 and allow to cool.

C Add 2-3 drops of ferroin indicator and titrate to a reddish-brown end point with the ferrous ammonium sulfate. Calculate the normality, N, of the ferrous ammonium sulfate.

D Calculation

$$\text{N of } Fe(NH_4)_2(SO_4)_2 = \frac{\text{ml } K_2Cr_2O_7 \times \text{N of } K_2Cr_2O_7}{\text{ml } Fe(NH_4)_2(SO_4)_2}$$

IV PROCEDURE

A Place 0.4 g $HgSO_4$ in a 500 ml 24/40 standard taper Erlenmeyer flask. Add 20 ml of sample, or an aliquot diluted to 20 ml with distilled water.

B Add 2 ml of concentrated H_2SO_4 and swirl to dissolve the $HgSO_4$.

C Add 10.0 ml of the 0.250 N $K_2Cr_2O_7$ and mix.

D Carefully add 28 ml of the sulfuric acid - silver sulfate reagent.

E Add several pumice granules or glass beads to prevent bumping, and then swirl the mixture to insure complete mixing.

F Reflux the mixture for two hours.

G Allow the solution to cool, wash down the condenser with distilled water, and add about 75 ml water to bring the volume to about 150 ml.

H Add 2-3 drops of the ferroin indicator and titrate the solution to a reddish-brown end point with the ferrous ammonium sulfate.

I A blank consisting of 20 ml of distilled water and containing all reagents is refluxed and titrated in the same manner as the sample.

J Calculation

$$\text{mg COD/l} = \frac{(A-B)\ N \times 8 \times 1000}{\text{ml of sample}}$$

COD = chemical oxygen demand

A = ml $Fe(NH_4)_2(SO_4)_2$ used for blank

B = ml $Fe(NH_4)_2(SO_4)_2$ used for sample

N = N of $Fe(NH_4)_2(SO_4)_2$

8 = equivalent weight of oxygen

ACKNOWLEDGEMENT:

Portions of this outline were taken from an outline prepared by R. J. Liska.

REFERENCES

1. Methods for Chemical Analysis of Water & Wastes, EPA-AQCL, 1971.
2. Standard Methods, APHA-AWWA-WPCF, 13th Edition, 1971.
3. ASTM Standards, Part 23, 1970.

This outline was prepared by Charles R. Feldmann, Chemist, EPA, WPO National Training Center, Cincinnati, OH 45268.

Descriptors: Chemical Analysis, Chemical Oxygen Demand, Organic Compounds, Oxidation, Oxygen, Oxygen Demand, Oxygen Requirements, Water Analysis

DISSOLVED OXYGEN

Determination By The Winkler Iodometric Titration and Azide Modification

The basic Winkler procedure (1888) has been modified many times to improve its workability in polluted waters. None of these modifications have been completely successful. The most useful modification was proposed by Alsterberg and consists of the addition of sodium azide to control nitrite interference during the iodometric titration. The Azide modification of the iodometric titration is recommended as official by the EPA-WPO Quality Control Committee for relatively clean waters.

A Reactions

1 The determination of DO involves a complex series of interactions that must be quantitative to provide a valid DO result. The number of sequential reactions also complicates interference control. The reactions will be presented first followed by discussion of the functional aspects.

$$MnSO_4 + 2\ KOH \rightarrow Mn(OH)_2 + K_2SO_4 \quad (a)$$

$$2\ Mn(OH)_2 + O_2 \rightarrow 2\ MnO(OH)_2 \quad (b)$$

$$MnO(OH)_2 + 2\ H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O \quad (c)$$

$$Mn(SO_4)_2 + 2\ KI \rightarrow MnSO_4 + K_2SO_4 + I_2 \quad (d)$$

$$I_2 + 2\ Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\ NaI \quad (e)$$

2 Reaction sequence

The series of reactions involves five different operational steps in converting dissolved oxygen in the water into a form in which it can be measured.

a $O_2 \rightarrow MnO(OH)_2 \rightarrow Mn(SO_4)_2 \rightarrow I_2 \rightarrow$ Thiosulfate (thio) or phenylarsine oxide (PAO) titration.

b All added reagents are in excess to improve contact possibilities and to force the reaction toward completion.

3 The first conversion, $O_2 \rightarrow MnO(OH)_2$ (reactions a, b) is an oxygen transfer operation where the dissolved oxygen in the water combines with manganous hydroxide to form an oxygenated manganic hydroxide.

a The manganous salt can react with oxygen only in a highly alkaline media.

b The manganous salt and alkali must be added separately with addition below the surface of the sample to minimize reaction with atmospheric oxygen via air bubbles or surface contact. Reaction with sample dissolved oxygen is intended to occur upon mixing of the reagents and sample after stoppering the full bottle (care should be used to allow entrained air bubbles to rise to the surface before adding reagents to prevent high results due to including entrained oxygen).

c Transfer of oxygen from the dissolved state to the precipitate form involves a two phase system of solution and precipitate requiring effective mixing for quantitative transfer. Normally a gross excess of reagents is used to limit mixing requirements. Mixing by rapid inversion 25 to 35 times will accomplish the purpose. Less energy is required by inversion 5 or 6 times, allowing the solids to settle half way and repeat the process. Reaction is rapid; contact is the principal problem in the two phase system.

d If the alkaline floc is white, no oxygen is present.

4 Acidification (reactions c and d) changes the oxygenated manganic hydroxide to manganic sulfate which in turn reacts with potassium iodide to form elemental iodine. Under acid conditions, oxygen cannot react directly with the excess manganous sulfate remaining in solution.

5 Iodine (reaction e) may be titrated with sodium thiosulfate standard solution to indicate the amount of dissolved oxygen originally present in the sample.

a The blue color of the starch-iodine complex commonly is used as an indicator. This blue color disappears when elemental iodine has been reacted with an equivalent amount of thiosulfate.

b Phenylarsine oxide solutions are more expensive to obtain but have better keeping qualities than thiosulfate solutions. Occasional use, field operations and situations where it is not feasible to calibrate thio solutions regularly, usually encourage use of purchased PAO reagents.

6 For practical purposes the DO determination scheme involves the following operations.

a Fill a 300 ml bottle* under conditions minimizing DO changes. This means that the sample bottle must be flushed with test solution to displace the air in the bottle with water characteristic of the tested sample*.

*DO test bottle volumes should be checked - discard those outside of the limits of 300 ml + or - 3 ml.

b To the filled bottle:

1) Add $MnSO_4$ reagent (2 ml)

2) Add KOH, KI, NaN_3 reagent (2 ml)
Stopper, mix by inversion, allow to settle half way and repeat the operation. Highly saline test waters commonly settle very slowly at this stage and may not settle to the half way point in the time allotted.

c To the alkaline mix (settled about half way) add 2 ml of sulfuric acid, stopper and mix until the precipitate dissolves.

d Transfer the contents of the bottle to a 500 ml Erlenmeyer flask and titrate with 0.0375 Normal Thiosulfate*. Each ml of reagent used represents 1 mg of DO/liter of sample.

The same thing applies for other sample volumes when using an appropriate titrant normality such as:

1) For a 200 ml sample, use 0.025 N Thio

2) For a 100 ml sample, use 0.0125 N Thio

*EPA-WPO Method

7 The addition of the first two DO reagents, ($MnSO_4$ and the KOH, KI and NaN_3 solutions) displaces an equal quantity of the sample. This is not the case when acid is added because the clear liquid above the floc does not contain dissolved oxygen as all of it should be converted to the particulate $MnO(OH)_2$. Some error is introduced by this displacement of sample during dosage of the first two reagents. The error upon addition of 2 ml of each reagent to a 300 ml sample is $\frac{4}{300} \times 100$ or 1.33% loss in DO. This may be corrected by an appropriate factor or by adjustment of reagent normality. It is generally considered small in relation to other errors in sampling, manipulation and interference, hence this error may be recognized but not corrected.

8 Reagent preparation and procedural details can be found in reference 1.

IV The sequential reactions for the Chemical DO determination provides several situations where significant interference may occur in application on polluted water, such as:

A Sampling errors may not be strictly designated as interference but have the same effect of changing sample DO. Inadequate flushing of the bottle contents or exposure to air may raise the DO of low oxygen samples or lower the DO of supersaturated samples.

B Entrained air may be trapped in a DO bottle by:

1 Rapid filling of vigorously mixed samples without allowing the entrained air to escape before closing the bottle and adding DO reagents.

2 Filling a bottle with low temperature water holding more DO than that in equilibrium after the samples warm to working temperature.

3 Aeration is likely to cool the sample permitting more DO to be introduced than can be held at the room or incubator temperatures.

4 Samples warmer than working incubator temperatures will be only partially full at equilibrium temperatures.

Addition of DO reagents results in reaction with dissolved or entrained oxygen. Results for DO are invalid if there is any evidence of gas bubbles in the sample bottle.

C The DO reagents respond to any oxidant or reductant in the sample capable of reacting within the time allotted. HOCl or H_2O_2 may raise the DO titration while H_2S, & SH^- may react with sample oxygen to lower the sample titration. The items mentioned react rapidly and raise or lower the DO result promptly. Other items such as Fe^{++} or $SO_3^{=}$ may or may not react completely within the time allotted for reaction. Many organic materials or complexes from benthic deposits may have an effect upon DO results that are difficult to predict. They may have one effect during the alkaline stage to release iodine from KI while favoring irreversible absorption of iodine during the acid stage. Degree of effect may increase with reaction time. It is generally inadvisable to use the iodometric titration on samples containing large amounts of organic contaminants or benthic residues. It would be expected that benthic residues would tend toward low results because of the reduced iron and sulfur content - they commonly favor high results due to other factors that react more rapidly, often giving the same effect as in uncontrolled nitrite interference during titration.

D Nitrite is present to some extent in natural waters or partially oxidized treatment plant samples. Nitrite is associated with a cyclic reaction during the acid stage of the DO determination that may lead to erroneous high results.

1 These reactions may be represented as follows:

$$\begin{array}{ll} \mathrm{2HNO_2 + 2\ HI \rightarrow I_2 + 4H_2O + N_2O_2} & \text{(a)} \\ \uparrow \qquad\qquad\qquad\qquad\qquad\qquad\qquad \downarrow & \\ \mathrm{2HNO_2 \leftarrow + 1/2O_2 + H_2O + N_2O_2} & \text{(b)} \end{array}$$

These reactions are time, mixing and concentration dependent and can be minimized by rapid processing.

2 Sodium azide ($\mathrm{NaN_3}$) reacts with nitrite under acid conditions to form a combination of $\mathrm{N_2 + N_2O}$ which effectively blocks the cyclic reaction by converting the $\mathrm{HNO_2}$ to noninterfering compounds of nitrogen.

3 Sodium azide added to fresh alkaline KI reagent is adequate to control interference up to about 20 mg of $\mathrm{NO_2^-}$ N/liter of sample. The azide is unstable and gradually decomposes. If resuspended benthic sediments are not detectable in a sample showing a returning blue color, it is likely that the azide has decomposed in the alkaline KI azide reagent.

E Surfactants, color and Fe+++ may confuse endpoint detection if present in significant quantities.

F Polluted water commonly contains significant interferences such as C. It is advisable to use a membrane protected sensor of the electronic type for DO determinations in the presence of these types of interference.

G The order of reagent addition and prompt completion of the DO determination is critical. Stable waters may give valid DO results after extended delay of titration during the acidified stage. For unstable water, undue delay at any stage of processing accentuates interference problems.

ACKNOWLEDGMENTS

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This outline was prepared by F. J. Ludzack, Chemist, National Training Center, EPA, WPO, Cincinnati, OH 45268, and revised by C. R. Feldmann, Chemist, National Training Center.

Descriptors: Chemical Analysis, Dissolved Oxygen, Oxygen, Water Analysis

DISSOLVED OXYGEN
DETERMINATION BY ELECTRONIC MEASUREMENT

I INTRODUCTION

A Electronic measurement of DO is attractive for several reasons:

1 Electronic methods are more readily adaptable for automated analysis, continuous recording, remote sensing or portability.

2 Application of electronic methods with membrane protection of sensors affords a high degree of interference control.

3 Versatility of the electronic system permits design for a particular measurement, situation or use.

4 Many more determinations per man-hour are possible with a minor expenditure of time for calibration.

B Electronic methods of analysis impose certain restrictions upon the analyst to insure that the response does, in fact, indicate the item sought.

1 The ease of reading the indicator tends to produce a false sense of security. Frequent and careful calibrations are essential to establish workability of the apparatus and validity of its response.

2 The use of electronic devices requires a greater degree of competence on the part of the analyst. Understanding of the behavior of oxygen must be supplemented by an understanding of the particular instrument and its behavior during use.

C Definitions

1 Electrochemistry - a branch of chemistry dealing with relationships between electrical and chemical changes.

2 Electronic measurements or electrometric procedures - procedures using the measurement of potential differences as an indicator of reactions taking place at an electrode or plate.

3 Reduction - any process in which one or more electrons are added to an atom or an ion, such as $O_2 + 2e \rightarrow 2O^-$ The oxygen has been reduced.

4 Oxidation - any process in which one or more electrons are removed from an atom or an ion, such as $Zn^o - 2e \rightarrow Zn^{+2}$. The zinc has been oxidized.

5 Oxidation - reduction reactions - in a strictly chemical reaction, reduction cannot occur unless an equivalent amount of some oxidizable substance has been oxidized. For example:

$2H_2 + O_2 \rightleftarrows 2H_2O$

$2H_2 - 4e \rightleftarrows 4H^{+1}$ hydrogen oxidized

$O_2 + 4e \rightleftarrows 2O^{-2}$ oxygen reduced

Chemical reduction of oxygen may also be accomplished by electrons supplied to a noble metal electrode by a battery or other energizer.

6 Anode - an electrode at which oxidation of some reactable substance occurs.

7 Cathode - an electrode at which reduction of some reactable substance occurs. For example in I. C. 3, the reduction of oxygen occurs at the cathode.

8 Electrochemical reaction - a reaction involving simultaneous conversion of chemical energy into electrical energy or the reverse. These conversions are

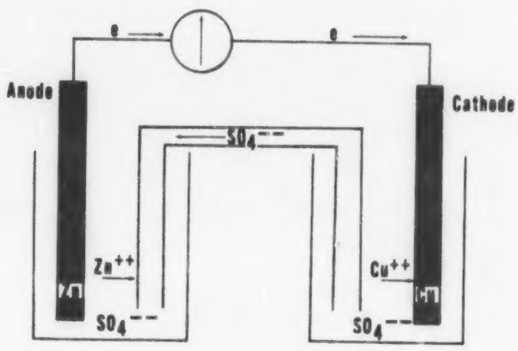
Note: Mention of Commercial Products and Manufacturers Does Not Imply Endorsement by the Environmental Protection Agency.

equivalent in terms of chemical and electrical energy and generally are reversible.

9 Electrolyte a solution, gel, or mixture capable of conducting electrical energy and serving as a reacting media for chemical changes. The electrolyte commonly contains an appropriate concentration of selected mobile ions to promote the desired reactions.

10 Electrochemical cell - a device consisting of an electrolyte in which 2 electrodes are immersed and connected via an external metallic conductor. The electrodes may be in separate compartments connected by tube containing electrolyte to complete the internal circuit.

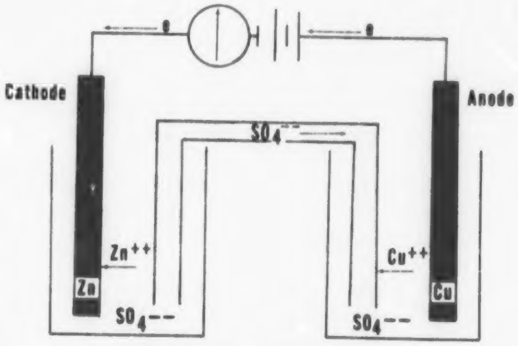
a Galvanic (or voltaic) cell - an electrochemical cell operated in such a way as to produce electrical energy from a chemical change, such as a battery (See Figure 1).



GALVANIC CELL

Figure 1

b Polarographic (electrolytic) cell - an electrochemical cell operated in such a way as to produce a chemical change from electrical energy (See Figure 2).



POLAROGRAPHIC CELL

Figure 2

D As indicated in I. C. 10 the sign of an electrode may change as a result of the operating mode. The conversion by the reactant of primary interest at a given electrode therefore designates terminology for that electrode and operating mode. In electronic oxygen analyzers, the electrode at which oxygen reduction occurs is designated the cathode.

E Each cell type has characteristic advantages and limitations. Both may be used effectively.

1 The galvanic cell depends upon measurement of electrical energy produced as a result of oxygen

reduction. If the oxygen content of the sample is negligible, the measured current is very low and indicator driving force is negligible, therefore response time is longer.

2 The polarographic cell uses a standing current to provide energy for oxygen reduction. The indicator response depends upon a change in the standing current as a result of electrons released during oxygen reduction. Indicator response time therefore is not dependent upon oxygen concentration.

3 Choice may depend upon availability, habit, accessories, or the situation. In each case it is necessary to use care and judgment both in selection and use for the objectives desired.

II ELECTRONIC MEASUREMENT OF DO

A Reduction of oxygen takes place in two steps as shown in the following equations:

1 $O_2 + 2H_2O + 2e \rightarrow H_2O_2 + 2OH^-$

2 $H_2O_2 + 2e^- \rightarrow 2OH^-$

Both equations require electron input to activate reduction of oxygen. The first reaction is more important for electronic DO measurement because it occurs at a potential (voltage) which is below that required to activate reduction of most interfering components (0.3 to 0.8 volts relative to the saturated calomel electrode - SCE). Interferences that may be reduced at or below that required for oxygen usually are present at lower concentrations in water or may be minimized by the use of a selective membrane or other means. When reduction occurs, a definite quantity of electrical energy is produced that is proportional to the quantity of reductant entering the reaction. Resulting current measurements thus are more specific for oxygen reduction.

B Most electronic measurements of oxygen are based upon one of two techniques for evaluating oxygen reduction in line with equation II.A.1. Both require activating energy, both produce a current proportional to the quantity of reacting reductant. The techniques differ in the means of supplying the activating potential; one employs a source of outside energy, the other uses spontaneous energy produced by the electrode pair.

1 The polarographic oxygen sensor relies upon an outside source of potential to activate oxygen reduction. Electron gain by oxygen changes the reference voltage.

a Traditionally, the dropping mercury electrode (DME) has been used for polarographic measurements. Good results have been obtained for DO using the DME but the difficulty of maintaining a constant mercury drop rate, temperature control, and freedom from turbulence makes it impractical for field use.

b Solid electrodes are attractive because greater surface area improves sensitivity. Poisoning of the solid surface electrodes is a recurrent problem. The use of selective membranes over noble metal electrodes has minimized but not eliminated electrode contamination. Feasibility has been improved sufficiently to make this type popular for regular use.

2 Galvanic oxygen electrodes consist of a decomposable anode and a noble metal cathode in a suitable electrolyte to produce activating energy for oxygen reduction (an air cell or battery). Lead is commonly used as the anode because its decomposition potential favors spontaneous reduction of oxygen. The process is continuous as long as lead and oxygen are in contact in the electrolyte and the electrical energy released at the cathode may be dissipated by an outside circuit. The anode may be conserved by limiting oxygen availability. Interrupting the outside circuit may produce erratic behavior for a time after reconnection. The resulting

current produced by oxygen reduction may be converted to oxygen concentration by use of a sensitivity coefficient obtained during calibration. Provision of a pulsed or interrupted signal makes it possible to amplify or control the signal and adjust it for direct reading in terms of oxygen concentration or to compensate for temperature effects.

III ELECTRONIC DO ANALYZER APPLICATION FACTORS

A Polarographic or galvanic DO instruments operate as a result of oxygen partial pressure at the sensor surface to produce a signal characteristic of oxygen reduced at the cathode of some electrode pair. This signal is conveyed to an indicating device with or without modification for sensitivity and temperature or other influences depending upon the instrument capabilities and intended use.

1 Many approaches and refinements have been used to improve workability, applicability, validity, stability and control of variables. Developments are continuing. It is possible to produce a device capable of meeting any reasonable situation, but situations differ.

2 Most commercial DO instruments are designed for use under specified conditions. Some are more versatile than others. Benefits are commonly reflected in the price. It is essential to determine the requirements of the measurement situation and objectives for use. Evaluation of a given instrument in terms of sensitivity, response time, portability, stability, service characteristics, degree of automation, and consistency are used for judgment on a cost/benefit basis to select the most acceptable unit.

B Variables Affecting Electronic DO Measurement

1 Temperature affects the solubility of oxygen, the magnitude of the resulting signal and the permeability of the protective membrane. A curve of oxygen solubility in water versus increasing temperature may be concave downward while a similar curve of sensor response versus temperature is concave upward. Increasing temperature decreases oxygen solubility and increases probe sensitivity and membrane permeability. Thermistor actuated compensation of probe response based upon a linear relationship or average of oxygen solubility and electrode sensitivity is not precisely correct as the maximum spread in curvature occurs at about 17° C with lower deviations from linearity above or below that temperature. If the instrument is calibrated at a temperature within + or - 5° C of working temperature, the compensated readout is likely to be within 2% of the real value. Depending upon probe geometry, the laboratory sensor may require 4 to 6% correction of signal per ° C change in liquid temperature.

2 Increasing pressure tends to increase electrode response by compression and contact effects upon the electrolyte, dissolved gases and electrode surfaces. As long as entrained gases are not contained in the electrolyte or under the membrane, these effects are negligible.

Inclusion of entrained gases results in erratic response that increases with depth of immersion.

3 Electrode sensitivity changes occur as a result of the nature and concentration of contaminants at the electrode surfaces and possible physical chemical or electronic side reactions produced. These may take the form of a physical barrier, internal short, high residual current, or chemical changes in the metal surface. The membrane is intended to allow dissolved gas penetration but to exclude passage of ions or particulates. Apparently some ions or materials producing extraneous ions within the electrode vicinity are able to pass in limited amounts which

become significant in time. Dissolved gases include 1) oxygen, 2) nitrogen, 3) carbon dioxide, 4) hydrogen sulfide, and certain others. Item 4 is likely to be a major problem. Item 3 may produce deposits in alkaline media; most electrolytes are alkaline or tend to become sc in line with reaction II.A.1. The usable life of the sensor varies with the type of electrode system, surface area, amount of electrolyte and type, membrane characteristics, nature of the samples to which the system is exposed and the length of exposure. For example, galvanic electrodes used in activated sludge units showed that the time between cleanup was 4 to 6 months for electrodes used for intermittent daily checks of effluent DO; continuous use in the mixed liquor required electrode cleanup in 2 to 4 weeks. Each electrometric cell configuration and operating mode has its own response characteristics. Some are more stable than others. It is necessary to check calibration frequency required under conditions of use as none of them will maintain uniform response indefinitely. Calibration before and after daily use is advisable.

4 Electrolytes may consist of solutions or gels of ionizable materials such as acids, alkalies or salts. Bicarbonates, KCl and KI are frequently used. The electrolyte is the transfer and reaction media, hence, it necessarily becomes contaminated before damage to the electrode surface may occur. Electrolyte concentration, nature, amount and quality affect response time, sensitivity, stability, and specificity of the sensor system. Generally a small quantity of electrolyte gives a shorter response time and higher sensitivity but also may be affected to a greater extent by a given quantity of contaminating substances.

5 Membranes may consist of teflon, polyethylene, rubber, and certain other polymeric films. Thickness may vary from 0.5 to 3 mils (inches × 1/1000). A thinner membrane will decrease response time and increase sensitivity but is less selective and may be ruptured more easily. The choice of material and its uniformity affects response time, selectivity and durability. The area of the membrane and its permeability are directly related to the quantity of transported materials that may produce a signal. The permeability of the membrane material is related to temperature and to residues accummulated on the membrane surface or interior. A cloudy membrane usually indicates deposition and more or less loss of signal.

6 Test media characteristics control the interval of usable life between cleaning and rejuvenation for any type of electrode. More frequent cleanup is essential in low quality waters than for high quality waters. Reduced sulfur compounds are among the more troublesome contaminants. Salinity affects the partial pressure of oxygen at any given temperature. This effect is small compared to most other variables but is significant if salinity changes by more than 500 mg/l.

7 Agitation of the sample in the vicinity of the electrode is important because DO is reduced at the cathode. Under quiescent conditions a gradient in dissolved oxygen content would be established on the sample side of the membrane as well as on the electrode side, resulting in atypical response. The sample should be agitated sufficiently to deliver a representative portion of the main body of the liquid to the outer face of the membrane. It is commonly observed that no agitation will result in a very low or negigible response after a short period of time. Increasing agitation will cause the response to rise gradually until some minimum liquid velocity is reached that will not cause a further increase in response with increased mixing energy. It is important to check mixing velocity to reach a stable high signal that is independent of a reasonable change in sample mixing. Excessive

mixing may create a vortex and expose the sensing surface to air rather than sample liquid. This should be avoided. A linear liquid velocity of about 1 ft/sec at the sensing surface is usually adequate.

8 DO sensor response represents a potential or current signal in the milli-volt or milli-amp range in a high resistance system. A high quality electronic instrument is essential to maintain a usable signal-to-noise ratio. Some of the more common difficulties include:

  a Variable line voltage or low batteries in amplifier power circuits.

  b Substandard or unsteady amplifier or resistor components.

  c Undependable contacts or junctions in the sensor, connecting cables, or instrument control circuits.

  d Inadequately shielded electronic components.

  e Excessive exposure to moisture, fumes or chemicals in the wrong places lead to stray currents, internal shorts or other malfunction.

C Desirable Features in a Portable DO Analyzer

1 The unit should include steady state performance electronic and indicating components in a convenient but sturdy package that is small enough to carry.

2 There should be provisions for addition of special accessories such as bottle or field sensors, agitators, recorders, line extensions, if needed for specific requirements. Such additions should be readily attachable and detachable and maintain good working characteristics.

3 The instrument should include a sensitivity adjustment which upon calibration will provide for direct reading in terms of mg of DO/liter.

4 Temperature compensation and temperature readout should be incorporated.

5 Plug in contacts should be positive, sturdy, readily cleanable and situated to minimize contamination. Water seals should be provided where necessary.

6 The sensor should be suitably designed for the purpose intended in terms of sensitivity, response, stability, and protection during use. It should be easy to clean, and reassemble for use with a minimum loss of service time.

7 Switches, connecting plugs, and contacts preferably should be located on or in the instrument box rather than at the "wet" end of the line near the sensor. Connecting cables should be multiple strand to minimize separate lines. Calibration controls should be convenient but designed so that it is not likely that they will be inadvertently shifted during use.

8 Agitator accessories for bottle use impose special problems because they should be small, self contained, and readily detachable but sturdy enough to give positive agitation and electrical continuity in a wet zone.

9 Major load batteries should be rechargeable or readily replaceable. Line operation should be feasible wherever possible.

10 Service and replacement parts availability are a primary consideration. Drawings, parts identification and trouble shooting memos should be incorporated with applicable operating instructions in the instrument manual in an informative organized form.

D Sensor and Instrument Calibration

The instrument box is likely to have some form of check to verify electronics, battery or other power supply conditions for use. The sensor commonly is not included in this check. A known reference sample used with the instrument in an operating mode is the best available method to compensate for sensor variables under use conditions. It is advisable to calibrate before and after daily use under test conditions. Severe conditions, changes in conditions, or possible damage call for calibrations during the use period. The readout scale is likely to be labeled - calibration is the basis for this label.

The following procedure is recommended:

1 Turn the instrument on and allow it to reach a stable condition. Perform the recommended instrument check as outlined in the operating manual.

2 The instrument check usually includes an electronic zero correction. Check each instrument against the readout scale with the sensor immersed in an agitated solution of sodium sulfite containing sufficient cobalt chloride to catalyze the reaction of sulfite and oxygen. The indicator should stabilize on the zero reading. If it does not, it may be the result of residual or stray currents, internal shorting in the electrode, or membrane rupture. Minor adjustments may be made using the indicator rather than the electronic controls. Serious imbalance requires electrode reconditioning if the electronic check is O.K. Sulfite must be carefully rinsed from the sensor until the readout stabilizes to prevent carry over to the next sample.

3 Fill two DO bottles with replicate samples of clarified water similar to that to be tested. This water should not contain significant test interferences.

4 Determine the DO in one by the azide modification of the iodometric titration.

5 Insert a magnetic stirrer in the other bottle or use a probe agitator. Start agitation after insertion of the sensor assembly and note the point of stabilization.

a Adjust the instrument calibration control if necessary to compare with the titrated DO.

b If sensitivity adjustment is not possible, note the instrument stabilization point and designate it as ua. A sensitivity coefficient, ϕ is equal to $\frac{ua}{DO}$ where DO is the titrated value for the sample on which ua was obtained. An unknown DO then becomes $DO = \frac{ua}{\phi}$. This factor is applicable as long as the sensitivity does not change.

6 Objectives of the test program and the type of instrument influence calibration requirements. Precise work may require calibration at 3 points in the DO range of interest instead of at zero and high range DO. One calibration point frequently may be adequate.

Calibration of a DO sensor in air is a quick test for possible changes in sensor response. The difference in oxygen content of air and of water is too large for air calibration to be satisfactory for precise calibration for use in water.

IV This section reviews characteristics of several sample laboratory instruments. Mention of a specific instrument does not imply EPA endorsement or recommendation. No attempt has been made to include all the available instruments; those described are used to indicate the approach used at one stage of development which may or may not represent the current available model.

A The electrode described by Carrit and Kanwisher (1) is illustrated in Figure 3. This electrode was an early example of those using a membrane. The anode was a silver - silver oxide reference cell with a platinum disc cathode (1-3 cm diameter). The salt bridge consisted of N/2 KCl and

KOH. The polyethylene membrane was held in place by a retaining ring. An applied current was used in a polarographic mode. Temperature effects were relatively large. Thermistor correction was studied but not integrated with early models.

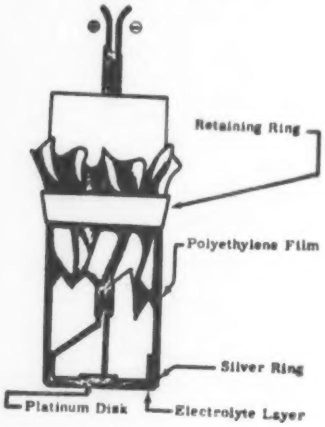

Retaining Ring
Polyethylene Film
Silver Ring
Platinum Disk
Electrolyte Layer

Figure 3

B The Beckman oxygen electrode is another illustration of a polarographic DO sensor (Figure 4). It consists of a gold cathode, a silver anode, an electrolytic gel containing KCl, covered by a teflon membrane. The instrument has a temperature readout and compensating thermistor, a source polarizing current, amplifier with signal adjustment and a readout DO scale with recorder contacts.

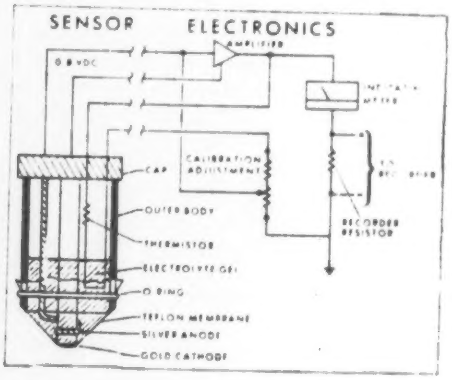

SENSOR
ELECTRONICS
AMPLIFIER
0.8 VDC
INDICATING METER
CALIBRATION ADJUSTMENT
TO RECORDER
RECORDER RESISTOR
CAP
OUTER BODY
THERMISTOR
ELECTROLYTE GEL
O RING
TEFLON MEMBRANE
SILVER ANODE
GOLD CATHODE

Figure 4. THE BECKMAN OXYGEN SENSOR

C The YSI Model 51 (3) is illustrated in Figure 5. This is another form of polarographic DO analyzer. The cell consists of a silver anode coil, a gold ring cathode and a KCl electrolyte with a teflon membrane. The instrument has a sensitivity adjustment, temperature and DO readout. The model 51 A has temperature compensation via manual preset dial. A field probe and bottle probe are available.

YSI Model 51 DO Sensor

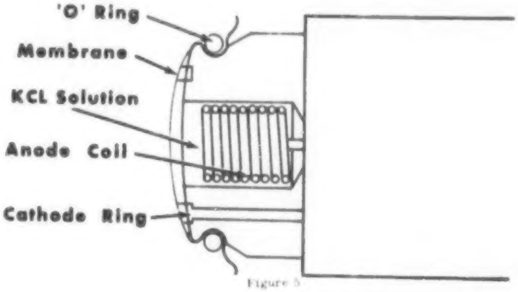
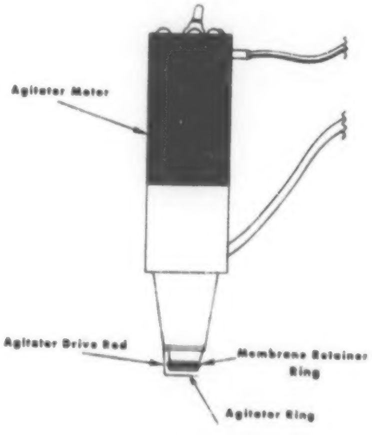

'O' Ring
Membrane
KCL Solution
Anode Coil
Cathode Ring

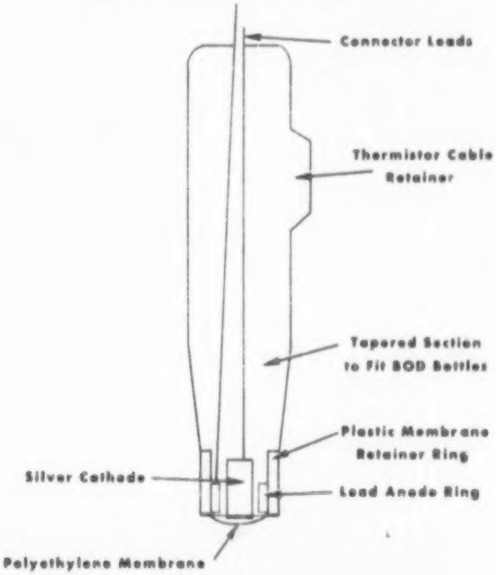
Figure 5

D The Model 54 YSI DO analyzer (4) is based upon the same electrode configuration but modified to include automatic temperature compensation, DO readout, and recorder jacks. A motorized agitator bottle probe is available for the Model 54 (Figure 6).

YSI Model 54 Agitator Probe


Agitator Motor
Agitator Drive Rod
Membrane Retainer Ring
Agitator Ring

E The Galvanic Cell Oxygen Analyzer (7, 8) employs an indicator for proportional DO signal but does not include thermistor compensation or signal adjustment. Temperature readout is provided. The sensor includes a lead anode ring, and a silver cathode with KOH electrolyte (4 molar) covered by a membrane film (Figure 7).


Connector Leads
Thermistor Cable Retainer
Tapered Section to Fit BOD Bottles
Plastic Membrane Retainer Ring
Silver Cathode
Lead Anode Ring
Polyethylene Membrane

Precision Galvanic Cell Oxygen Probe

Figure 7

F The Weston and Stack Model 300 DO Analyzer (8) has a galvanic type sensor with a pulsed current amplifier adjustment to provide for signal and temperature compensation. DO and temperature readout is provided. The main power supply is a rechargeable battery. The sensor (Figure 8) consists of a lead anode coil recessed in the electrolyte cavity (50% KI) with a platinum cathode in the tip. The sensor is covered with a teflon membrane. Membrane retention by rubber band or by a plastic retention ring may be used for the bottle agitator or depth sampler respectively. The thermistor and agitator are mounted in a sleeve that also provides protection for the membrane.

G The EIL Model 15 A sensor is illustrated in Figure 9. This is a galvanic cell with thermistor activated temperature compensation and readout. Signal adjustment is provided. The illustration shows an expanded scheme of the electrode which when assembled compresses into a sensor approximating 5/8 inch diameter and 4 inch length exclusive of the enlargement at the upper end. The anode consists of compressed lead shot in a replaceable capsule (later models used fine lead wire coils), a perforated silver cathode sleeve around the lead is covered by a membrane film. The electrolyte is saturated potassium bicarbonate. The large area of lead surface, silver and membrane provides a current response of 200 to 300 microamperes in oxygen saturated water at 20° C for periods of up to 100 days use (8). The larger electrode displacement favors a scheme described by Eden (9) for successive DO readings for BOD purposes.

V Table 1 summarizes major characteristics of the sample DO analyzers described in Section IV. It must be noted that an ingenious analyst may adapt any one of these for special purposes on a do-it-yourself program. The sample instruments are mainly designed for laboratory or portable field use. Those designed for field monitoring purposes may include similar designs or alternate designs generally employing larger anode, cathode, and electrolyte capacity to approach better response stability with some sacrifice in response time and sensitivity. The electronic controls, recording, telemetering, and accessory apparatus generally are semi-permanent installations of a complex nature.

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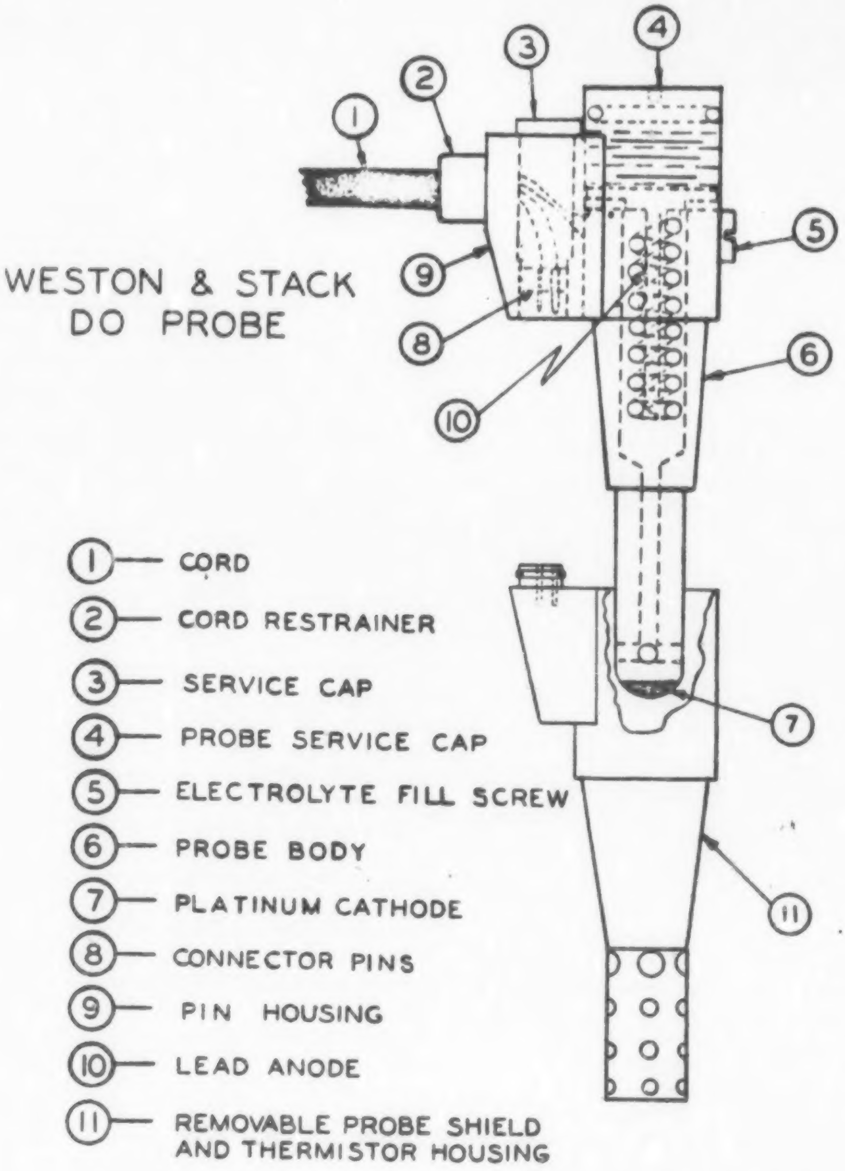
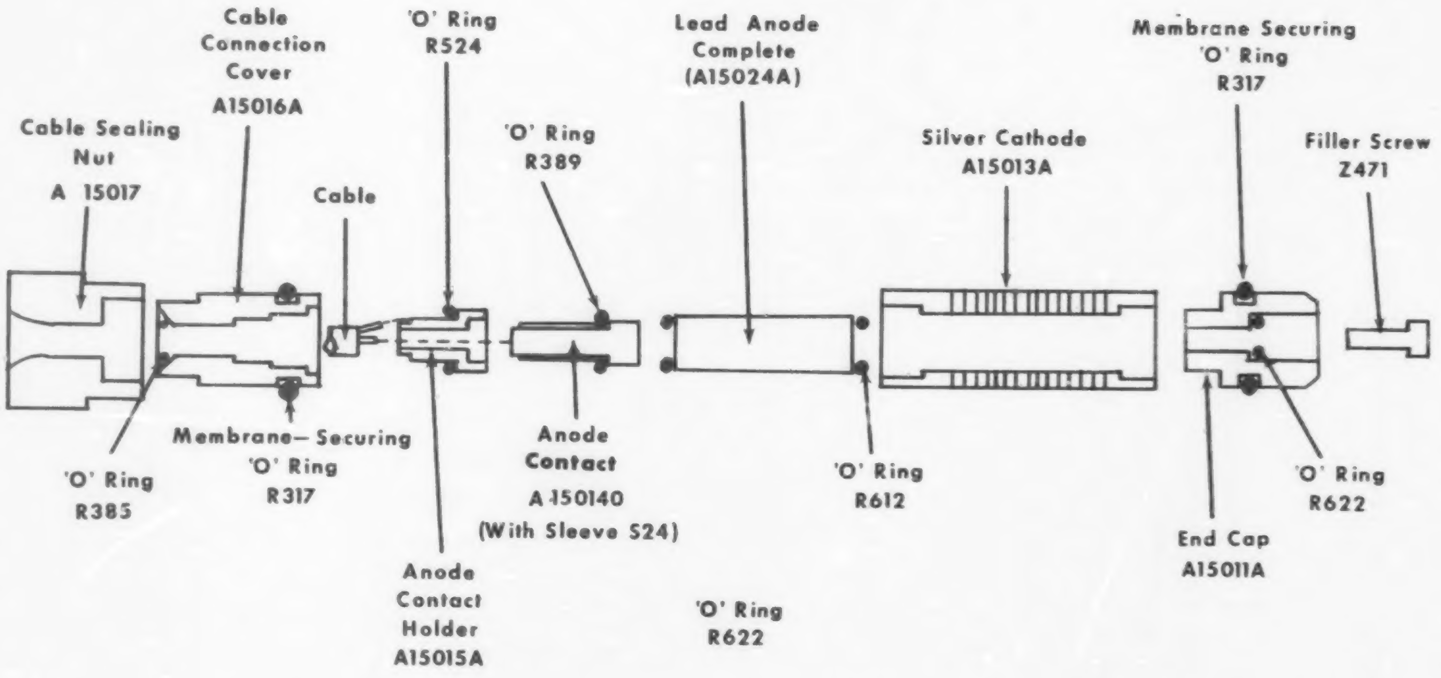


WESTON & STACK
DO PROBE

1 — CORD
2 — CORD RESTRAINER
3 — SERVICE CAP
4 — PROBE SERVICE CAP
5 — ELECTROLYTE FILL SCREW
6 — PROBE BODY
7 — PLATINUM CATHODE
8 — CONNECTOR PINS
9 — PIN HOUSING
10 — LEAD ANODE
11 — REMOVABLE PROBE SHIELD AND THERMISTOR HOUSING

Figure 8

Model A15A ELECTRODE COMPONENT PARTS


Cable Sealing Nut A 15017
Cable Connection Cover A15016A
Cable
'O' Ring R524
'O' Ring R389
Lead Anode Complete (A15024A)
Silver Cathode A15013A
Membrane Securing 'O' Ring R317
Filler Screw Z471
'O' Ring R385
Membrane— Securing 'O' Ring R317
Anode Contact Holder A15015A
Anode Contact A 150140 (With Sleeve S24)
'O' Ring R622
'O' Ring R612
End Cap A15011A
'O' Ring R622

Note: Red wire of cable connects to Anode Contact Holder

Black wire of cable connects to Anode Contact

Membrane not shown E. I. L. part number T22

Figure 9

TABLE 1

CHARACTERISTICS OF VARIOUS LABORATORY DO INSTRUMENTS

	Anode	Cathode	Elec	Type	Membr	DO Sig. Adj.	Temp. Comp. Temp. Rdg.	Accessories for which designed
Carrit & Kanwisher	silver-silver ox. ring	Pt disc	KCl KOH N/2	pol*	polyeth	no	no	Recording temp. & signal adj. self assembled
Beckman	Aq ring	Au disc	KCl gel	pol	teflon	yes	yes yes	recording
Yellow Springs 51	Ag coil	Au ring	KCl soln sat.	pol	teflon	yes	no* yes	field and bottle probe
Yellow Springs 54	"	"	"	"	"	yes	yes yes	recording field bottle & agitator probes
Precision Sci	Pb ring	silver disc	KOH 4N	galv**	polyeth	no	no yes	
Weston & Stack 300	Pb coil	Pt disc	KI 40%	galv	teflon	yes	yes yes	agit. probe depth sampler
EIL	Pb	Ag	$KHCO_3$	galv	teflon	yes	yes yes	recording
Delta 75	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes no	field bottle & agitator probe
Delta 85	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes yes	field bottle & agitator probe

*Pol - Polarographic (or amperometric)

**Galv - Galvanic (or voltametric)

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This outline was prepared by F. J. Ludzack Chemist, EPA, WPO, National Training Center, Cincinnati, OH 45268 and Nate Malof, Chemist, EPA, WPO, National Field Investigations Center, Cincinnati, OH

Descriptors: Chemical Analysis, Dissolved Oxygen, Dissolved Oxygen Analyzers, Instrumentation, On-Site Tests, Water Analysis

SOURCES, EFFECTS, CLASSIFICATION & DETERMINATION OF PHOSPHORUS IN THE AQUEOUS ENVIRONMENT

I Phosphorus is closely associated with water quality because of (a) its role in aquatic productivity such as algal blooms, (b) its sequestering action, which causes interference in coagulation, (c) the difficulty of removing phosphorus from water to some desirable low concentration, and (d) its characteristic of converting from one to another of many possible forms.

A Phosphorus is one of the primary nutrients such as hydrogen (H), carbon (C), nitrogen (N), sulfur (S) and phosphorus (P).

1 Phosphorus is unique among nutrients in that its oxidation does not contribute significant energy because it commonly exists in oxidized form.

2 Phosphorus is intimately involved in oxidative energy release from and synthesis of other nutrients into cell mass via:

a Transport of nutrients across membranes into cell protoplasm is likely to include phosphorylation.

b The release of energy for metabolic purposes is likely to include a triphosphate exchange mechanism.

B Most natural waters contain relatively low levels of P (0.01 to 0.05 mg/l) in the soluble state during periods of significant productivity.

1 Metabolic activity tends to convert soluble P into cell mass (organic P) as a part of the protoplasm, intermediate products, or sorbed material.

2 Degradation of cell mass and incidental P compounds results in a feedback of lysed P to the water at rates governed by the type of P and the environment. Aquatic metabolic kinetics show marked influences of this feedback.

3 The concentrations of P in hydrosoils, sludges, treatment plant samples and soils may range from 10^2 to 10^6 times that in stabilized surface water. Both concentration and interfering components affect applicability of analytical techniques.

II The primary source of phosphorus in the aqueous system is of geological origin. Indirect sources are the processed mineral products for use in agriculture, household, industry or other activities.

A Agricultural fertilizer run-off is related to chemicals applied, farming practice and soil exchange capacity.

B Wastewaters primarily of domestic origin contain major amounts of P from:

1 Human, animal and plant residues

2 Surfactants (cleaning agent) discharge

3 Microbial and other cell masses

C Wastewaters primarily of industrial origin contain P related to:

1 Corrosion control

2 Scale control additives

3 Surfactants or dispersants

4 Chemical processing of materials including P

5 Liquors from clean-up operations of dusts, fumes, stack gases, or other discharges

III Phosphorus terminology is commonly confused because of the interrelations among biological, chemical, engineering, physical, and analytical factors.

A Biologically, phosphorus may be available as a nutrient, synthesized into living mass, stored in living or dead cells, agglomerates, or mineral complexes, or converted to degraded materials.

B Chemically, P exists in several mineral and organic forms that may be converted from one to another under favorable conditions. Analytical estimates are based upon physical or chemical techniques necessary to convert various forms of P into ortho phosphates which alone can be quantitated in terms of the molybdenum blue colorimetric test.

C Engineering interest in phosphorus is related to the prediction, treatment, or control of aqueous systems to favor acceptable water quality objectives. Phosphorus removal is associated with solids removal.

D Solubility and temperature are major physical factors in phosphorus behavior. Soluble P is much more available than insoluble P for chemical or biological transformations and the rate of conversion from one to another is strongly influenced by temperature.

E Table 1 includes a classification of the four main types of chemical P and some of the relationships controlling solubility of each group. It is apparent that no clear-cut separation can be made on a solubility basis as molecular weight, substituent and other factors affect solubility.

F Table 2 includes a scheme of analytical differentiation of various forms of P based upon:

  1 The technique required to convert an unknown variety of phosphorus into ortho P which is the only one quantitated by the colorimetric test.

  2 Solubility characteristics of the sample P or more precisely the means required to clarify the sample.

    a Any clarification method is subject to incomplete separation. Therefore, it is essential to specify the method used to interpret the yield factor of the separation technique. The degree of separation of solubles and insolubles will be significantly different for:

      1 Membrane filter separation (0.5 micron pore size)

      2 Centrifugation (at some specified rpm and time)

      3 Paper filtration (specify paper identification)

      4 Subsidence (specify time and conditions)

G Analytical separations (Table 2) like those in Table 1, do not give a precise separation of the various forms of P which may be included quantitatively with ortho or poly P. Conversely some of the poly and organic P will be included with ortho P if they have been partially hydrolyzed during storage or analysis. Insolubles may likewise be included as a result of poor separation and analytical conditions.

  1 The separation methods provide an operational type of definition adequate in most situations if the "operation" is known. Table 2 indicates the nature of incidental P that may appear along with the type sought.

Table 1

PHOSPHORUS COMPOUNDS CLASSIFIED BY CHEMICAL AND SOLUBILITY RELATIONS

Form	Water Soluble(1)	Insoluble(1)
1. Ortho phosphates $(PO_4)^{-3}$	Combined with monovalent cations such as H^+, Na^+, K^+, NH_4^+	Combined with multi valent cations such as Ca^{+2} Al^{+3} Fe^{+3}
2. Poly phosphates $(P_2O_7),^{-4}(P_3O_{10})^{-5}(P_3O_9)^{-3}$ and others depending upon the degree of dehydration.	as in 1 above Increasing dehydration decreases solubility	(a) as in 1 above (b) multi P polyphosphates (high mol. wt.) including the "glassy" phosphates
3. Organic phosphorus R-P, R-P-R (2) (unusually varied nature)	(a) certain chemicals (b) degradation products (c) enzyme P (d) phosphorylated nutrients	(a) certain chemicals (b) cell mass, may be colloidal or agglomerated (c) soluble P sorbed by insoluble residues
4. Mineral phosphorus	(a) as in 1 above	(a) as in 1 above (b) as in 2 above (c) geological P such as phosphosilicates (d) certain mineral complexes.

(1) Used in reference to predominance under common conditions.

(2) R represents an organic radical, P represents P, PO_4^{-3}, or its derivatives.

---

2 Total P in Table 2 includes liquid and separated residue P that may exist in the whole sample including silt, organic sludge, or hydrosoils. This recognizes that the feedback of soluble P from deposited or suspended material has a real effect upon the kinetics of the aqueous environment.

Table 2

PHOSPHORUS COMPOUNDS CLASSIFIED BY ANALYTICAL METHODOLOGY

Desired P Components	Technique(1)	Incidental P Included(2)
1. Ortho phosphates	No treatment on clear samples	Easily hydrolyzed (a) poly phosphates - (b) organic -P, - (c) Mineral -P, + or -
2. Polyphosphates (2)-(1) = poly P (hydrolyzable)	acid hydrolysis on clear samples, dilute (a) H_2SO_4 (b) HCl heated	(a) ortho-P + (b) organic -P + or - (c) mineral -P + or -
3. Organic phosphorus (3) - (2) + org P (hydrolyzable)	acid + oxidizing hydrolysis on whole sample. dilute (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + (NH_4)_2S_2O_8$ heated	(a) ortho P + (b) poly P + (c) mineral P + or -
4. Soluble phosphorus (preferably classified by clarification method)	clarified liquid following filtration, centrifugation or subsidence	generally includes (a) 1, 2, or 3 (b) particulates not completely separated
5. Insoluble phosphorus (residue from clarification)	Retained residues separated during clarification See (6)	(a) generally includes sorbed or complexed solubles.
6. Total phosphorus	Strong acid + oxidant digestion (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + HNO_3 + HClO_4$ (c) $H_2O_2 + Mg(NO_3)_2$ fusion	all components in 1, 2, 3, 4, 5 in the whole sample

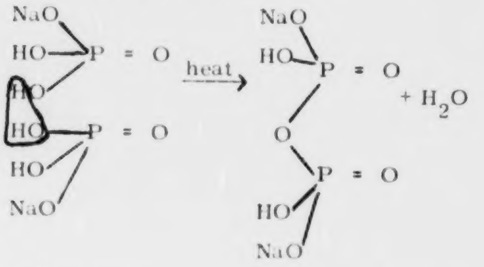
(1) Determinative step by phospho molybdate colorimetric method.

(2) Coding: + quantitative yield
- a small fraction of the amount present
+ or - depends upon the individual chemical and sample history

IV Polyphosphates are of major interest in cleaning agent formulation, as dispersants, and for corrosion control.

A They are prepared by dehydration of ortho phosphates to form products having two or more phosphate derivatives per molecule.

1 The simplest polyphosphate may be prepared as follows:



mono sodium ortho phosphate (2)

disodium dihydrogen polyphosphate

2 The general form for producing polyphosphates from mono substituted orthophosphates is:

$$n\ (NaH_2PO_4)\ \xrightarrow[200-400^{\circ}C]{heat}\ (NaPO_3)_n + n\ H_2O$$

3 Di-substituted ortho phosphates or mixtures of substituted ortho phosphates lead to other polyphosphates:

$$2Na_2HPO_4 + NaH_2PO_4 \xrightarrow{heat} Na_5P_3O_{10} + 2H_2O$$

disodium hydrogen ortho phosphate + mono sodium di hydrogen ortho phosphate → pentasodium tri-phosphate + water

4 The polyphosphate series usually consist of the polyphosphate anion with a negative charge of 2 to 5. Hydrogen or metals commonly occupy these sites. The polyphosphate can be further dehydrated by heat as long as hydrogen remains. Di or trivalent cations generally produce a more insoluble polyphosphate than the same cation in the form of insoluble ortho phosphate. Insolubility increases with the number of P atoms in the polyphosphate. The "glassy" polyphosphates are a special group with limited solubility that are used to aid corrosion resistance in pipe distribution systems and similar uses.

B Polyphosphates tend to hydrolyze or "revert" to the ortho P form by addition of water. This occurs whenever polyphosphates are found in the aqueous environment.

1 The major factors affecting the rate of reversion of poly to orthophosphates include:

a) Temperature, increased T increases rate

b) pH, lower pH increases rate

c) Enzymes, hydrolase enzymes increase rate

d) Colloidal gels, increase rate

e) Complexing cations and ionic concentration increase rate

f) Concentration of the polyphosphate increases rate

2 Items a, b and c have a large effect upon reversion rate compared with other factors listed. The actual reversion rate is a combination of listed items and other conditions or characteristics.

3 The differences among ortho and ortho + polyphosphates commonly are close to experimental error of the colorimetric test in stabilized surface water samples. A significant difference generally indicates that the sample was obtained relatively close to a source of polyphosphates and was promptly analyzed. This implies that the reversion rate of polyphosphates is much higher than generally believed.

V SAMPLING AND PRESERVATION TECHNIQUES

A Sampling

1 Great care should be exercised to exclude any benthic deposits from water samples.

2 Glass containers should be acid rinsed before use.

3 Certain plastic containers may be used. Possible adsorption of low concentrations of phosphorus should be checked.

4 If a differentiation of phosphorus forms is to be made, filtration should be carried out immediately upon sample collection. A membrane filter of 0.45 μ pore size is recommended for reproducible separations.

B Preservation

1 If at all possible, samples should be analyzed on the day of collection. At best, preservation measures only retard possible changes in the sample.

a Possible physical changes include solubilization, precipitation, absorption on or desorption from suspended matter.

b Possible chemical changes include reversion of poly to ortho P and decomposition of organic or mineral P.

c Possible biological changes include microbial decomposition of organic P and algal or bacterial growth forming organic P.

2 Refrigeration at 4°C is recommended if samples are to be stored. This decreases hydrolysis and reaction rates and also losses due to volatility.

3 Addition of 40 mg $HgCl_2$/liter is recommended for longer storage periods. This chemical limits biological changes.

a $HgCl_2$ is an interference in the analytical procedure if the chloride level is low (See Part VI, B3).

VI THE EPA ANALYTICAL PROCEDURE(6)

A This is a colorimetric determination, specific for orthophosphate. Depending on the nature of the sample and on the type of data sought, the procedure involves two general operations:

1 Conversion of phosphorus forms to soluble orthophosphate (See Fig. 1):

a sulfuric acid-hydrolysis for polyphosphates, and some organic P compounds.

b persulfate digestion for organic P compounds.

2 The color determination involves reacting dilute solutions of phosphorus with ammonium molybdate and potassium antimonyl tartrate in an acid medium to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the orthophosphate concentration.

Color absorbance is measured at 880 nm and a concentration value obtained using a standard curve.

Reagent preparation and the detailed procedure can be found in the EPA manual.

The methods described there are usable in the 0.01 to 0.5 mg/liter phosphorus range.

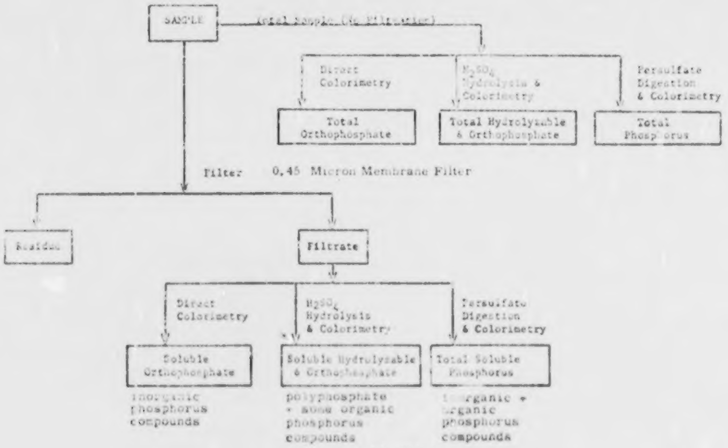

SAMPLE
Total Sample (No Filtration)
Direct Colorimetry
H_2SO_4 Hydrolysis & Colorimetry
Persulfate Digestion & Colorimetry
Total Orthophosphate
Total Hydrolyzable & Orthophosphate
Total Phosphorus
Filter
0.45 Micron Membrane Filter
Residue
Filtrate
Direct Colorimetry
H_2SO_4 Hydrolysis & Colorimetry
Persulfate Digestion & Colorimetry
Soluble Orthophosphate
Soluble Hydrolyzable & Orthophosphate
Total Soluble Phosphorus
inorganic phosphorus compounds
polyphosphate + some organic phosphorus compounds
inorganic + organic phosphorus compounds

FIGURE 1

ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS

B Interferences

1 Erroneous results from contaminated glassware is avoided by cleaning it with hot 1:1 HC1, treating it with procedure reagents and rinsings with distilled water. Preferably this glassware should be used only for the determination of phosphorus and protected from dust during storage. Commercial detergents should never be used.

2 High iron concentrations in samples can precipitate phosphorus.

3 If $HgC1_2$ is used as a preservative, it interferes if the chloride level of the sample is less than 50 mg C1/liter. Spiking with NaC1 is then recommended.

4 Others have reported interference from chlorine, chromium, sulfides, nitrite, tannins, lignin and other minerals and organics at high concentrations.

C Precision and Accuracy$^{(6)}$

1 Organic phosphate - 33 analysts in 19 laboratories analyzed natural water samples containing exact increments of organic phosphate of 0.110, 0.132, 0.772, and 0.882 mg P/liter.

Standard deviations obtained were 0.033, 0.051, 0.130 and 0.128 respectively.

Accuracy results as bias, mg P/liter were: +0.003, +0.016, +0.023 and - 0.008, respectively.

2 Orthophosphate was determined by 26 analysts in 16 laboratories using samples containing orthophosphate in amounts of 0.029, 0.038, 0.335 and 0.383 mg P/liter.

Standard deviations obtained were 0.010, 0.008, 0.018 and 0.023 respectively.

Accuracy results as bias, mg P/liter were -0.001, -0.002, -0.009 and -0.007 respectively.

VII VARIABLES IN THE COLORIMETRIC PROCEDURE

Several important variables affect formation of the yellow heteropoly acid and its reduced form, molybdenum blue, in the colorimetric test for P.

A Acid Concentration during color development is critical. Figure 2 shows that color will appear in a sample containing no phosphate if the acid concentration is low. Interfering color is negligible when the normality with respect to H_2SO_4 approaches 0.4.

1 Acid normality during color development of 0.3 to slightly more than 0.4 is feasible for use. It is preferable to control acidity carefully and to seek a normality closer to the higher limits of the acceptable range.

2 It is essential to add the acid and molybdate as one solution.

3 The aliquot of sample must be neutralized prior to adding the color reagent.

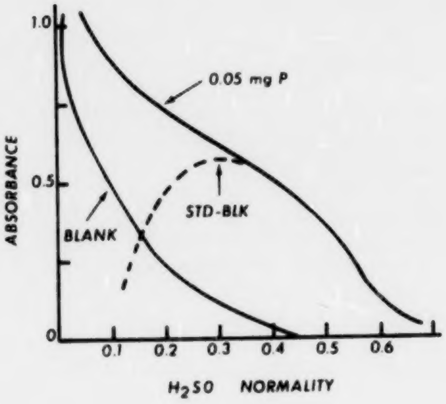


Figure 2

0-PHOSPHATE COLOR VS ACIDITY

B Choice of Reductant - Reagent stability, effective reduction and freedom from deleterious side effects are the bases for reductant selection. Several reductants have been used effectively. Ascorbic acid reduction is highly effective in both marine and fresh water. It is the reductant specified in the EPA method.

C Temperature affects the rate of color formation. Blank, standards, and samples must be adjusted to the same temperature (± 1°C), (preferably room temperature), before addition of the acid molybdate reagent.

D Time for Color Development must be specified and consistent. After addition of reductant, the blue color develops rapidly for 10 minutes then fades gradually after 12 minutes.

VIII DETERMINATION OF TOTAL PHOSPHORUS

A Determination of total phosphorus content involves omission of any filtration procedure and using the acid-hydrolysis and persulfate treatments to convert all phosphorus forms to the test-sensitive orthophosphate form.

B Determining total phosphorus content yields the most meaningful data since the various forms of phosphorus may change from one form to another in a short period of time. (See part V, B1)

IX DEVELOPMENT OF A STANDARD PROCEDURE

Phosphorus analysis received intensive investigation; coordination and validation of methods is more difficult than changing technique.

A Part of the problem in methods arose because of changes in analytical objectives such as:

1 Methods suitable to gather "survey" information may not be adequate for "standards".

2 Methods acceptable for water are not necessarily effective in the presence of significant mineral and organic interference characteristic of hydrosoils, marine samples, organic sludges and benthic deposits.

3 Interest has been centered on "fresh" water, it was essential to extend them for marine waters.

4 Instrumentation and automation have required adaptation of methodology.

B Analysts have tended to work on their own special problems. If the method apparently served their situations, it was used. Each has a "favorite" scheme that may be quite effective but progress toward widespread application of "one" method has been slow. Consequently, many methods are available. Reagent acidity, Mo content, reductant and separation techniques are the major variables.

C At the present time there is not sufficient data to warrant EPA endorsement of the P procedure for sediment-type samples, sludges, algal blooms, etc. Following is a procedure (not included in the EPA manual) which is useful when solids are present in samples:

1 If sample contains large particles, grind and emulsify solids in a blender.

2 Transfer 50 ml sample, or aliquot diluted to 50 ml, into a 250 ml Erlenmeyer flask.

3 Add 6.0 ml of 18N H_2SO_4, 5 ml concentrated HNO_3, 2 berl saddles and digest on hot plate.

4 Digest until the disappearance of nitric acid fumes and the appearance of white SO_3 fumes. Continue digestion for approximately 5 minutes. Cool before proceeding with Step 5.

5 Add 2 ml of HNO_3-$HClO_4$ mixture and 5 ml concentrated HNO_3. Continue digestion until all of the nitric acid is driven off and dense fumes of perchloric evolve. Perchloric acid requires dilution with sulfuric acid and prior destruction of most organics for safety.

6 Cool. Add approximately 40 ml distilled water and transfer to 100 ml volumetric flask.

7 Add 2-3 drops of phenolphthalein and concentrated ammonium hydroxide until a pink color is seen. Then discharge the pink color with the strong sulfuric acid. It is advisable to add an equivalent amount of salt formed during neutralization of digested samples to the calibration standards to equalize salt content during color development.

8 Determine orthophosphate according to the usual color procedure.

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This outline was prepared by Audrey E. Donahue, Chemist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

Descriptors:
Chemical Analysis, Nutrients, Phosphates, Phosphorus, Phosphorus Compounds, Pollutant Identification, Sampling, Water Analysis, Water Pollution Sources.

LABORATORY PROCEDURE FOR NITRATE NITROGEN

MODIFIED BRUCINE METHOD

(Range 0.1 to 2 mg nitrate nitrogen/l)

I REAGENTS

A Water

Distilled water free of nitrite and nitrate should be used in the preparation of all reagents and standards.

B Stock Potassium Nitrate (1.0 ml = 0.1 mg NO_3^- N):

Dissolve 0.7218 g of anhydrous KNO_3 in distilled water and dilute to 1 liter.

C Standard Potassium Nitrate (1.0 ml = 0.001 mg NO_3^- N):

Dilute 10.0 ml of the stock solution to 1 liter with distilled water. This solution should be prepared fresh weekly.

D Sulfuric Acid:

Carefully add 500 ml of concentrated H_2SO_4 (sp. gr. 1.84) to 125 ml of distilled water. Cool and keep tightly stoppered.

E Sodium Chloride (30%):

Dissolve 30 g of NaCl in distilled water and dilute to 100 ml.

F Brucine - sulfanilic acid reagent:

Dissolve 1 g of brucine sulfate, $(C_{23}H_{26}N_2O_4)_2 \cdot H_2SO_4 \cdot 7\,H_2O$, and 0.1 g of sulfanilic acid ($NH_2C_6H_4SO_3H \cdot H_2O$) in 70 ml of hot distilled water. Add 3 ml of concentrated HCl, cool, and dilute to 100 ml. This solution is stable for several months if stored in a dark bottle at 5°C. The pink color which develops slowly does not effect the usefulness of the solution. The bottle should be marked "toxic".

G Acetic Acid:

Dilute 1 vol of glacial $HC_2H_3O_2$ with 3 vols of distilled water.

II SAMPLE PREPARATION

A In the case of highly alkaline waters, it is necessary to adjust the pH to approximately 7 with the acetic acid.

B Filter through a 0.45 micron pore size filter if necessary.

III PROCEDURE

A Pipet 0.0, 2.0, 5.0, 7.0, and 10.0 ml of the standard KNO_3 solution into 50 ml test tubes held in a suitable rack. Add sufficient distilled water to bring the volume to 10.0 ml.

B Pipet 2.0, 3.0, and 5.0 ml. of the sample into 50 ml test tubes. The purpose of using three different volumes of the sample is to ensure that when the colors are developed, at least one of the three samples will give an absorbance which lies within the range of the calibration curve. Add sufficient distilled water to bring the volume to 10 ml.

C If the samples are saline, pipet 2.0 ml of 30% NaCl into the standard and sample tubes. Mix well. This addition is unnecessary for fresh water samples.

D Chill all of the tubes to 0 - 10°C in a cold water bath.

E Pipet 10.0 ml of the H_2SO_4 solution into each standard and sample tube, mix well by swirling, and again chill to 0 - 10°C.

F Pipet 0.5 ml of the brucine - sulfanilic acid reagent into the standard and sample tubes, mix well by swirling, and place the tubes in a boiling water bath for 25 minutes.

G Remove the tubes from the boiling water bath and cool to 20-25° C in a water bath.

H If color or turbidity develops in the sample while the tubes are in the boiling water bath, a blank must be prepared using all reagents except the brucine - sulfanilic acid.

I Measure the absorbance values of the standards and samples at 410 nm. on a suitable spectrophotometer. Before each solution is read, rinse the spectrophotometer cell at least twice with the solution.

J Prepare a calibration curve of absorbance values for the standards vs. mg of NO_3^- N. For example: if 2.0 ml of the standard KNO_3 solution are used, and its concentration is 0.001 mg of NO_3^- N/ml, then 0.002 mg of NO_3^- is the value plotted on the calibration curve vs. the corresponding absorbance value.

K Determine the mg of NO_3^- N present in the samples using the calibration curve and then calculate the mg of NO_3^- N/l using the formula:

$$\text{mg of } NO_3^- \text{ N/l} =$$

$$\text{mg of } NO_3^- \text{ N from curve} \times \frac{1000 \text{ ml/l}}{\text{ml of sample}}$$

REFERENCES

Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, 1971.

This outline was prepared by Charles R. Feldmann, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268

Descriptors:

Chemical Analysis, Nitrates, Nitrogen Compounds, Water Analysis

CHEMICAL TESTS,OBSERVATIONS,AND MEASUREMENTS IN THE FIELD

I INTRODUCTION

A Laboratory determinations with approved equipment and convenient facilities by experienced specialists generally are easier, faster, and more reliable. It is advisable to transport the samples to the laboratory whenever it is feasible to do so.

B Field tests are essential because:

1 Certain sample components are inherently unstable with respect to biological, chemical, or physical changes. Any test result performed in the laboratory may not represent true conditions on site at the time because of delay, displacement, or changed conditions.

2 Subsequent operations generally may be coordinated and made more meaningful by preliminary on site field investigation to identify and evaluate problems, locate critical areas, and minimize surprises.

II FIELD TEST CONDITIONAL CONSIDERATIONS

A Moving the laboratory into the field means improvisation and adaptation to more "primitive" conditions.

1 Rugged construction of field equipment is a first consideration. Sturdy and convenient cases are required; the case often may be the only available work-bench. Polyethylene bottles, beakers, burets, and pipets generally are necessary to eliminate breakage of fragile glass.

2 Portability is essential, particularly when the site is not accessible by boat or car. Ideally, the field kit should be small enough and light enough to be carried by one man for extended periods.

3 Procedures for field use are restricted in equipment and manipulation. In general, it is not possible to use long, tedious routines or highly precise measurements. Numerous reagents generally cannot be carried. Quick positive reactions are essential. Small visual comparators, test papers or spot tests are popular. Titration assemblies must be modified for field use.

4 Instrumentation increases objectivity of the measurements but the instruments must be adapted to the items outlined in II. A. 1, 2 and 3.

The instruments commonly used must be simplified yet rugged enough to keep working in spite of temperature changes, moisture, bumps, etc. A meaningful validation procedure must be worked out for calibration on site with simple adjustment or repair for emergencies.

5 Personnel engaged in field testing often are unaccustomed to laboratory procedures. Advance training is essential in procedures and instrumentation used. Field testing is a multi-disciplinarian operation:

a The individuals must be good observers and recognize what may be significant later.

b They must be good interpreters of a variety of observed and derived test information.

c They must be good technicians to follow prescribed procedures diligently and recognize anomalous behavior when it occurs.

d They must be good reporters to describe what happened, where it happened, and when. Failure to report anomalous behavior and related events in an understandable and consistent fashion may render the entire effort meaningless because of some doubt or inconsistency. Subsequent operations also may be mislead by the early observer.

B Use of field data imposes certain constraints relatable to the item, the methods, and to the personnel involved:

1 How valid is it?

2 How consistent is it in line with other observed or recorded information?

3 What backup information is required to verify suggested information?

C Decisions must be made on all field data applications:

1 Where "in-place" and "now" measurements are required how much of the laboratory must be moved out to the site to obtain data reliable enough to meet objectives?

2 Which items are to be determined on site? Which items in a central laboratory?

3 What use is to be made of the preliminary field estimates?

4 What do you "need" to know?

5 What would be "nice" to know?

6 In line with facilities, time, cost, manpower and objectives, what can you determine?

III COMPONENT CHARACTERISTICS FAVORING FIELD TESTING:

A Any item that changes rapidly during a holding period as a result of temperature or pressure variations, or is highly reactive from a biological, chemical, or physical standpoint, usually means that it must be determined on site and in place with minimum time lapse and manipulation.

1 Dissolved gases, such as O_2, CO_2, H_2S, Cl_2, are sensitive to pressure or temperature changes and may react with other sample components readily. These require in place and now readout.

2 The collective analysis of all forms of a given item may be preserved and analyzed later. Estimates of different forms of the same substance are not subject to delay unless they can be separated promptly. Examples of this include the relative ratios of oxidized or reduced substances, such as Cu^{+1} and Cu^{+2}, Fe^{+2} and Fe^{+3}, Cr^{+3} and Cr^{+6}; hydrolysis is the principal factor in the ratios of organic and NH_3-N and in changes among organic, poly and ortho-P.

3 Reactive substances contributing to "oxygen demand" tests, such as BOD or other respiratory tests generally require a minimum time delay before testing and are not amenable to preservation without altering results.

4 Soluble/insoluble ratios commonly change with time and conditions due to complexation, hydrolysis precipitation, agglomeration, or other factors. Particulate size may increase or decrease. Solubles may be sorbed on or desorbed from solid surfaces. Settleability or turbidity are transient phenomena subject to change with time or conditions.

5 Biological progressions are dynamic entities that shift among "critters" in relation to predominence in variety, numbers, growth, and decay. Whatever is, will change in response to nutrition and conditions.

IV SITUATIONS FAVORING FIELD TESTING

A Preliminary information often is needed to guide situation evaluation, problem identification, on site variation with respect to cross section, depth, or time. These data are useful to determine whether there is a problem or not and for planning of subsequent operations.

1 It may be necessary to locate suspected inflows, channels, or sources of items affecting water quality.

2 Definition of mixing zones often is required.

3 Laboratory time may be reduced if approximate concentrations of items sought are known.

4 Field tests or observations may suggest other tests that are more critical in evaluation of the given situation.

5 Stratification may be evaluated for guidance of subsequent operations.

6 Knowledge of the distribution of components is useful for selecting meaningful future sampling sites.

B The field test may be used to check compliance with regulations prior to more rigorous backup testing.

1 An established operation may be in control or out of control.

2 The field test may reveal which of multiple discharges are in conformance.

3 Undisclosed discharges may become apparent.

4 It may be necessary to "track" some hazardous discharge down stream to guide subsequent users on the choice of intake or storage water.

5 A complaint or inquiry may be evaluated by field tests.

C In-plant field type tests are essential to guide operations toward the production of continuous high quality effluents. Record tests commonly are provided too late to do anything about the situation; quick test results are emphasized for process control because time, manpower, and change are critical.

1 Surprises in the form of wastewater changes in flow, concentration, or significant components are common for treatment process operators. They need quick numbers rather than impressions to distinguish real from apparent problems.

2 Process upsets are minimized or prevented by regular and meaningful tests from which trends may be established. Small imbalances may be corrected before they become major upsets.

3 Backup or supplementary treatment such as coagulation, adsorption, neutralization, may prevent serious process disruption if tests indicate the problem and its magnitude in time to do something about it.

V TYPICAL FIELD TESTS - INSTRUMENTAL

A pH is one of the simplest and most valuable field tests. If the result is due to mineral acids or alkalies in stable form, color indicators or comparators, impregnated paper or other devices will serve. When free CO_2 in solution is a major variable such as in the vicinity of benthic deposits or in active biological systems, the application of an electronic sensor in-place and extension wire to an indicator instrument is essential. Any delay, pressure change or manipulation will change free CO_2 and alter pH response. Electronic pH instruments are produced for field use by many apparatus supply firms. With care, they will function effectively.

B Conductivity is a very useful index of ionic materials and usually may be correlated with wet chemical data to give a reasonable correlation with total dissolved solids for a given mixture. This test is very useful to detect saline intrustions or discharges, springs, hidden channels of different salinity from the main body of water. Several reliable instruments are marketed.

C DO analyzers are available for field use application from several manufacturers. They differ in portability accessory equipment and in versatility so that it is possible to obtain one or more fitting almost any requirement. These units are based upon reduction of oxygen at the cathode surface to convert chemical to electrical energy. Direct measurement of electrical energy produced or the change in some carrier current or voltage may be used for readout. The signal may be amplified in line with sensitivity to give direct concentration readout. Temperature compensation is useful and available. Membrane covered sensors selective for dissolved gases protect sensor surfaces from most interfering components. The analyst must learn to use his particular instrument effectively to enable him to obtain valid results under conditions in which a wet chemical procedure would be misleading.

VI TYPICAL FIELD TESTS - WET CHEMICAL

A DO titrations for clean water samples may be adapted for field use by shifting to dry reagents encased in plastic pillows, substituting phenyl arsene oxide for thio and using plastic burets, sample containers, etc. Powdered starch substitutes also are available. Sample sizes may be altered along with reagent concentration to maintain the 1 to 1 ratio of DO to titrant volume. Hach, La Motte and probably others, provide DO test kits packaged for field use. "Home made" kits are common.

B Alkalinity and hardness titrations are commonly sought under field conditions using standard acid and EDTA solutions. Methyl purple or mixed indicators commonly are used in place of methyl orange indicator because of better visibility in the field. Powder pillows for buffer and indicator are available for the hardness test.

C Chlorine tests using ortho tolidine in a comparator, such as those by Hilger or Taylor and others, frequently are used for rapid on site measurements. The comparators, reagents and comparator tubes commonly are available from apparatus, equipment, or chlorine suppliers.

VII SUMMARY

Simple and rapid field test kits are available from several manufacturers, suppliers and scientific apparatus houses for individual tests, as well as combination test kits for the items mentioned and others.

It is possible for the analyst to devise one suited to his particular requirements with available or improvised materials. Effectiveness of the field operation depends on the knowledge and care exercised in using the equipment and procedure.

CAUTION: Field test results are useful, but not always valid enough for the record.

ACKNOWLEDGEMENT:

This outline contains significant material from a previous outline by D. G. Ballinger.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, WPO, Cincinnati, OH 45268.

Descriptors: Chemical Analysis, On-Site Tests, Water Analysis

CHEMICAL FIELD KIT DESCRIPTION

I INTRODUCTION

Attached find Figure 1 of the kit. This kit is capable of measuring DO, temperature, alkalinity (both phenolphthalein and total) and hardness under field conditions. An approximation of pH can be obtained with indicators (phenolphthalein and methyl purple) and indicator papers contained in the kit. It is presumed that the chemist will also carry a portable pH meter, conductivity probe, and DO sampler, if desired. The tests can be performed with the following precision:

	Range	Precision ± 1 s
Dissolved Oxygen	0 - 10 mg/l	± 0.2 mg/l
Phenolphthalein Alkalinity	0 - 10 mg/l	± 1 mg/l
Total Alkalinity	100 - 300 mg/l	± 7 mg/l
Hardness	200 - 400 mg/l	± 15 mg/l

II MATERIALS USED

The kit is constructed of high grade outdoor plywood, reinforced with waterproof joints, and painted with epoxy paint. Styrofoam packing is used inside the case (See Figure 4); a polyurethane foam rectangular block (15 1/4" × 7 1/2") is placed inside the top lid to secure the reagents and equipment during travel. The pipets are held in two sponge rubber blocks. (See Figure 3)

III FUNCTION AND SET-UP OF EACH REAGENT AND PIECE OF EQUIPMENT (See Figure 2)

A Distilled Water (250 ml polyethylene bottle with wash bottle attachment). To be used for rinsing buret used in titrations.

B Manganous Sulfate (powder pillows available from Hach Chemical Company, Ames, Iowa).

C Alkaline-Iodide-Azide (available from Hach Chemical Company, Ames, Iowa).

D Sulfamic Acid. This powdered reagent can be used instead of sulfuric acid in the DO test. It is available as packaged powder pillows from Hach Chemical Company.

E 0.0250 N Phenylarsene Oxide, PAO (available from Hach Chemical Company) contained in a 250 ml polyethylene bottle with a wash bottle attachment. Add 90 ml of distilled water to 160 ml of the 0.0250 N PAO. This gives 250 ml of 0.016 N PAO.

F Starch (in a small 100 ml bottle - polyethylene). Superlose 500 - a soluble starch in powder form - is used. It is available from Stein Hall and Company, 285 Madison Avenue, New York, N.Y. $25/100 lb.

G Glass Stoppered DO Bottle (125 ml nominal size, 129 ml actual size)

This bottle is used to collect the DO sample. Reagents are directly added

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Office of Water Programs, Environmental Protection Agency.

and then entire sample is transferred to 250 ml Erlenmeyer flask for titration. The bottle also has calibration marks at 25, 50, 75, and 100 ml for use to obtain samples for other determinations.

H 250 ml Centrifuge Bottle or Erlenmeyer Flask (Polycarbonate). To be used for titrations.

I Phenolphthalein and methyl purple (in 100 ml polyethylene dropper bottles). Methyl purple can be obtained from Fleisher Chemical Company, Benjamin Franklin Station, Washington, D.C. 20204.

J 0.02 N Sulfuric Acid (in 250 ml polyethylene bottle with wash bottle attachment).

K Univer III (Hardness Indicator + Buffer) powder pillows. These are available from Hach Chemical Company.

L pH Testing Paper (Range 2.0 - 12.0 and 6.6 - 8.1). These papers are available in strip form from Oxyphen Products Company, P.O. Box 148, Forest Hills, New York.

M EDTA (0.01 M) - in 250 ml polyethylene bottle with wash bottle attachment.

N 10 ml pipet, buret tubing, microburet clamp, right angle clamp, scissors and 14 1/2" long aluminum rod. (See Figure 5)

O Thermometer - a pocket type of Weston all metal type.

P Chemical Kits and Reagents are also available from other companies such as LaMotte Chemical Company, Chestertown, Maryland.

Q Acidity Reagents can be conveniently substituted in the kit.

R If it is desirable, the kit can be manipulated so that it is possible to do more DO determinations. This is done by substituting DO bottles (125 ml) for either or both alkalinity or hardness reagents.

IV DIRECTIONS

A Dissolved Oxygen

1 Collect sample in 125 ml bottle.

2 Add 1 pillow manganous sulfate and 1 pillow alkaline iodide.

3 Stopper bottle. Mix. Allow precipitate to settle. Repeat step 3.

4 Add 1 pillow sulfamic acid. Mix well.

5 Transfer entire contents of 125 ml bottle to Erlenmeyer flask.

6 Titrate to pale yellow with 0.016 N PAO. At this point, add a pinch of starch.

7 Finish titration to colorless endpoint.

8 Calculation:

ml PAO used = mg oxygen/1

B Hardness

1 Measure 25 ml of sample into Erlenmeyer flask.

2 Add 1 indicator-buffer pillow.

3 Add a pinch of hardness indicator.

4 Titrate to blue endpoint with EDTA (1 ml = 1 mg $CaCO_3$). Note: The last few drops of the titration should be added at 3 sec. intervals.

5 Calculation:

mg/l hardness as $CaCO_3$ = EDTA used × 40

C Phenolphthalein Alkalinity

1 Measure 50 ml sample into Erlenmeyer flask.

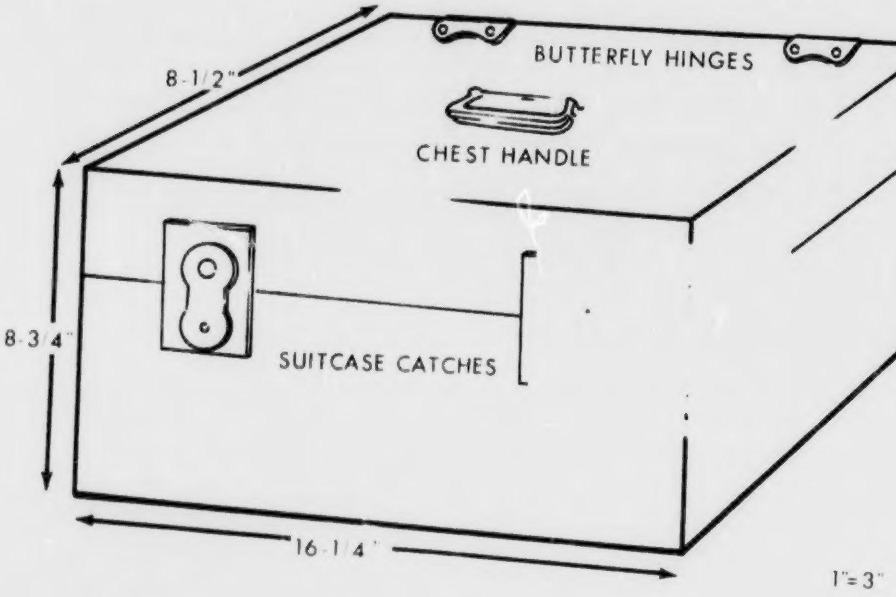
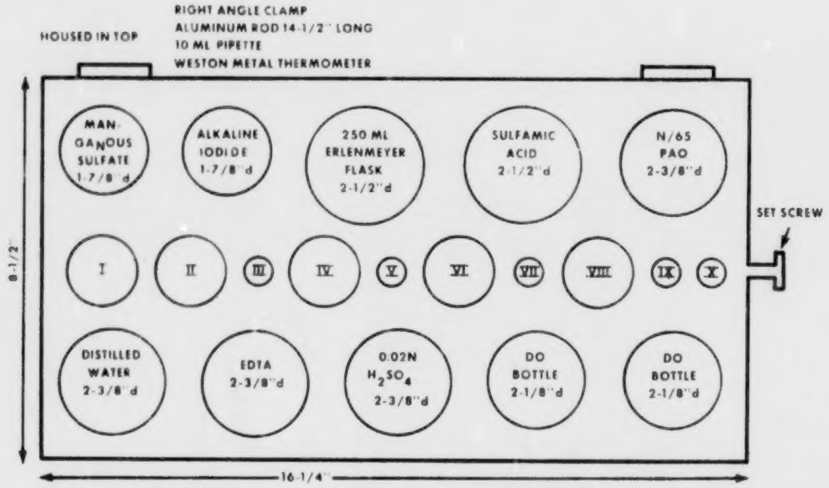

BUTTERFLY HINGES
CHEST HANDLE
8-1/2"
8-3/4"
SUITCASE CATCHES
16-1/4"
1"= 3"

Figure 1. VIEW OF CASE


HOUSED IN TOP
RIGHT ANGLE CLAMP
ALUMINUM ROD 14-1/2" LONG
10 ML PIPETTE
WESTON METAL THERMOMETER
MAN-GANOUS SULFATE 1-7/8"d
ALKALINE IODIDE 1-7/8"d
250 ML ERLENMEYER FLASK 2-1/2"d
SULFAMIC ACID 2-1/2"d
N/65 PAO 2-3/8"d
SET SCREW
I
II
III
IV
V
VI
VII
VIII
IX
X
8-1/2"
DISTILLED WATER 2-3/8"d
EDTA 2-3/8"d
0.02N H2SO4 2-3/8"d
DO BOTTLE 2-1/8"d
DO BOTTLE 2-1/8"d
16-1/4"

I	(1-1/4"d	pH TEST PAPER
II	(1-1/4"d	pH TEST PAPER
III	(1/2"d)	SCISSORS
IV	(1-3/8"d)	METHYL PURPLE
V	(1/2"d)	BURET TUBING
VI	(1-3/8"d)	PHENOLPHTHALEIN
VII	(1/2"d)	THERMOMETER
	(1-3/8"d)	STARCH
IX	(1/2"d)	BURET CLAMP
X	(3/8"d)	HOLE FOR ALUMINUM ROD (HELD IN PLACE WITH SET SCREW)

Figure 2. PLAN VIEW

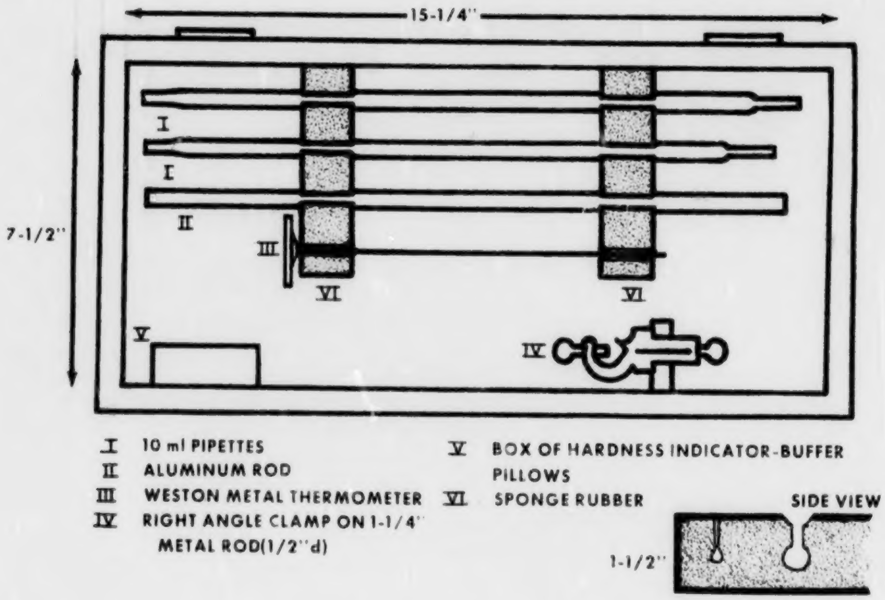

15-1/4"
7-1/2"
I
I
II
III
VI
VI
V
IV
I 10 ml PIPETTES
II ALUMINUM ROD
III WESTON METAL THERMOMETER
IV RIGHT ANGLE CLAMP ON 1-1/4" METAL ROD(1/2"d)
V BOX OF HARDNESS INDICATOR-BUFFER PILLOWS
VI SPONGE RUBBER
SIDE VIEW
1-1/2"

Figure 3. INSIDE TOP LID

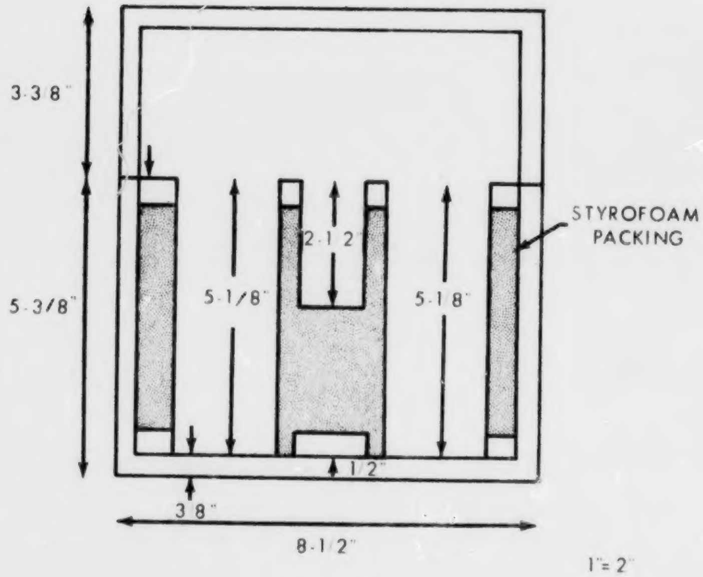

3-3/8"
5-3/8"
5-1/8"
2-1/2"
5-1/8"
STYROFOAM PACKING
1/2"
3/8"
8-1/2"
1"= 2"

Figure 4. TRANSVERSE SECTION

46 4

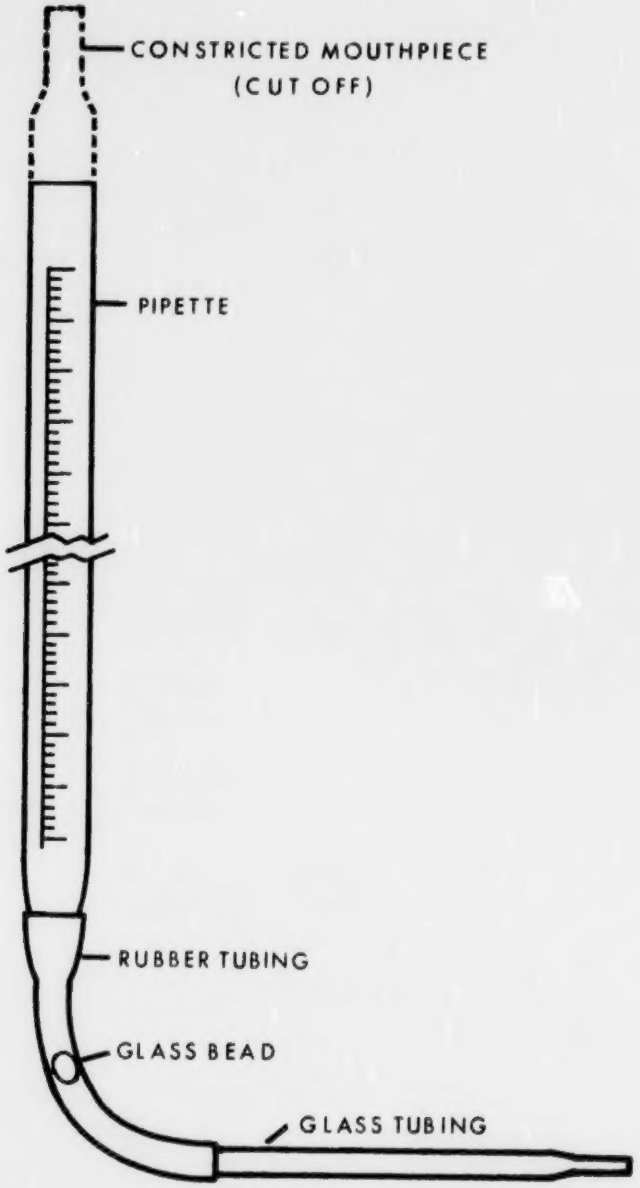

CONSTRICTED MOUTHPIECE
(CUT OFF)
PIPETTE
RUBBER TUBING
GLASS BEAD
GLASS TUBING

Figure 5. BURET

2 Add 2 drops phenolphthalein indicator.

3 If solution is pink, titrate to colorless endpoint with 0.02 N H_2SO_4.

4 Calculation:

mg/l phenolphthalein alkalinity as $CaCO_3$ =

ml 0.02 N H_2SO_4 × 20

D Total Alkalinity

1 To sample (on which phenolphthalein alkalinity has just been determined) add 5 drops methyl purple indicator.

2 Titrate from green color to purple endpoint with 0.02 N H_2SO_4.
NOTE: A blue-grey color appears just before the endpoint.

mg/l total alkalinity as $CaCO_3$ = mg/l

phenolphthalein alkalinity + mg/l methyl

purple alkalinity (ml 0.02 N H_2SO_4 used in methyl purple titration × 20).

REFERENCES

1 Kroner, Robert C., Longbottom, James E., and Gorman, Richard. A Comparison of Various Reagents Proposed for Use in the Winkler Procedure for Dissolved Oxygen. PHS Water Pollution Surveillance System Applications and Development Report #12. July 1964.

2 Water Minerals #3. Analytical Reference Service. Training Program, Robert A. Taft Sanitary Engineering Center. November 1961.

This outline was prepared by B. A. Punghorst, Chemist, formerly with National Training Center, FWQA, Cincinnati, OH 45226.

Descriptors: Alkalinity, Chemical Analysis, Dissolved Oxygen, Hardness, On-Site Tests, Testing, Water Analysis

BACTERIOLOGICAL INDICATORS OF WATER POLLUTION

Part 1. General Concepts

I INTRODUCTION

A Bacterial Indication of Pollution

1 In the broadest sense, a bacterial indicator of pollution is any organism which, by its presence, would demonstrate that pollution has occurred, and often suggest the source of the pollution.

2 In a more restrictive sense, bacterial indicators of pollution are associated primarily with demonstration of contamination of water, originating from excreta of warm-blooded animals (including man, domestic and wild animals, and birds).

B Implications of Pollution of Intestinal Origin

1 Intestinal wastes from warm-blooded animals regularly include a wide variety of genera and species of bacteria. Among these the coliform group may be listed, and species of the genera *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Proteus*, *Pseudomonas*, certain spore-forming bacteria, and others.

2 In addition, many kinds of pathogenic bacteria and other microorganisms may be released in wastes on an intermittent basis, varying with the geographic area, state of community health, nature and degree of waste treatment, and other factors. These may include the following:

a Bacteria: Species of *Salmonella*, *Shigella*, *Leptospira*, *Brucella*, *Mycobacterium*, and *Vibrio comma*.

b Viruses: A wide variety, including that of infectious hepatitis, Polioviruses, Coxsackie virus, ECHO viruses (enteric cytopathogenic human orphan -- "viruses in search of a disease"), and unspecified viruses postulated to account for outbreaks of diarrheal and upper respiratory diseases of unknown etiology, apparently infective by the water-borne route.

c Protozoa: *Endamoeba histolytica*

3 As routinely practiced, bacterial evidence of water pollution is a test for the presence and numbers of bacteria in wastes which, by their presence, indicate that intestinal pollution has occurred. In this context, indicator groups discussed in subsequent parts of this outline are as follows:

a Coliform group and certain subgroupings

b Fecal streptococci and certain subgroupings

c Miscellaneous indicators of pollution

4 Evidence of water contamination by intestinal wastes of warm-blooded animals is regarded as evidence of health hazard in the water being tested.

II PROPERTIES OF AN IDEAL INDICATOR OF POLLUTION

A An "ideal" bacterial indicator of pollution should:

1 Be applicable in all types of water

2 Always be present in water when pathogenic bacterial constituents of fecal contamination are present. Ramifications of this include --

  a Its density should have some direct relationship to the degree of fecal pollution.

  b It should have greater survival time in water than enteric pathogens, throughout its course of natural disappearance from the water body.

  c It should disappear rapidly from water following the disappearance of pathogens, either through natural or man-made processes.

  d It always should be absent in a bacteriologically safe water.

3 Lend itself to routine quantitative testing procedures without interference or confusion of results due to extraneous bacteria

4 Be harmless to man and other animals

B In all probability, an "ideal" bacterial indicator does not exist. The discussion of bacterial indicators of pollution in the following parts of this outline include consideration of the merits and limitations of each group, with their applications in evaluating bacterial quality of water.

III APPLICATIONS OF TESTS FOR POLLUTION INDICATORS

A Tests for Compliance with Bacterial Water Quality Standards

1 Potability tests on drinking water to meet Interstate Quarantine or other standards of regulatory agencies.

2 Determination of bacterial quality of environmental water for which quality standards may exist, such as shellfish waters, recreational waters, water resources for municipal or other supplies.

3 Tests for compliance with established standards in cases involving the protection or prosecution of municipalities, industries, etc.

B Treatment Plant Process Control

1 Water treatment plants

2 Wastewater treatment plants

C Water Quality Surveys

1 Determination of intestinal pollution in surface water to determine type and extent of treatment required for compliance with standards

2 Tracing sources of pollution

3 Determination of effects on bacterial flora, due to addition of organic or other wastes

D Special Studies, such as

1 Tracing sources of intestinal pathogens in epidemiological investigations

2 Investigations of problems due to the Sphaerotilus group

3 Investigations of bacterial interference to certain industrial processes, with respect to such organisms as Pseudomonas, Achromobacter, or others

IV SANITARY SURVEY

The laboratory bacteriologist is not alone in evaluation of indication of water pollution of intestinal origin. On-site study (Sanitary Survey) of the aquatic environment and adjacent areas, by a qualified person, is a necessary collateral study with the laboratory work and frequently will reveal information regarding potential bacteriological hazard which may or may not be demonstrated through laboratory findings from a single sample or short series of samples.

Part 2. The Coliform Group and Its Constituents

I ORIGINS AND DEFINITION

A Background

1 In 1885, Escherich, a pioneer bacteriologist, recovered certain bacteria from human feces, which he found in such numbers and consistency as to lead him to term these organisms "the characteristic organism of human feces."

He named these organisms Bacterium coli-commune and B. lactis aerogenes. In 1895, another bacteriologist, Migula, renamed B. coli commune as Escherichia coli, which today is the official name for the type species.

2 Later work has substantiated much of the original concept of Escherich, but has shown that the above species are in fact a heterogeneous complex of bacterial species and species variants.

a This heterogeneous group occurs not only in human feces but representatives also are to be found in many environmental media, including sewage, surface freshwaters of all categories, in and on soils, vegetation, etc.

b The group may be subdivided into various categories on the basis of numerous biochemical and other differential tests that may be applied.

B Composition of the Coliform Group

1 Current definition

As defined in "Standard Methods for the Examination of Water and Wastewater" (13th ed): "The coliform group includes all of the aerobic and facultative anaerobic, Gram-negative, nonspore-forming rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35° C."

2 The term "coliforms" or "coliform group" is an inclusive one, including the following bacteria which may meet the definition above:

a Escherichia coli, E. aurescens, E. freundii, E. intermedia

b Aerobacter aerogenes, A. cloacae

c Biochemical intermediates between the genera Escherichia and Aerobacter

* The above terminology is in accordance with the current editions of Standard Methods and Bergey's Manual of Determinative Bacteriology and will be consistent throughout this manual until these sources are modified.

3 There is no provision in the definition of coliform bacteria for "atypical" or "aberrant" coliform strains.

a An individual strain of any of the above species may fail to meet one of the criteria of the coliform group.

b Such an organism, by definition, is not a member of the coliform group, even though a taxonomic bacteriologist may be perfectly correct in classifying the strain in one of the above species.

II SUBDIVISION OF COLIFORMS INTO "FECAL" AND "NONFECAL" CATEGORIES

A Need

Single-test differentiations between coliforms of "fecal" origin and those of "nonfecal" origin are based on the assumption that typical E. coli and closely related strains are of fecal origin while A. aerogenes and its close relatives are not of direct fecal origin. (The latter assumption is not fully borne out by investigations at this Center. See Table 1, IMViC Type --++). A number of single differential tests have been proposed to differentiate between "fecal" and "nonfecal" coliforms.

Without discussion of their relative merits, several may be cited here:

B Types of Single-Test Differentiation

1 Determination of gas ratio

Fermentation of glucose by E. coli results in gas production, with hydrogen and carbon dioxide being produced in equal amounts. Fermentation of glucose by A. aerogenes results in generation of twice as much carbon dioxide as hydrogen.

Further studies suggested absolute correlation between H_2/CO_2 ratios and the terminal pH resulting from glucose fermentation. This led to the substitution of the methyl red test.

2 Methyl red test

Glucose fermentation by E. coli typically results in a culture medium having terminal pH in the range 4.2 - 4.6 (red color a positive test with the addition of methyl red indicator). A. aerogenes typically results in a culture medium having pH 5.6 or greater (yellow color, a negative test).

3 Indole

When tryptophane, an amino acid, is incorporated in a nutrient broth, typical E. coli strains are capable of producing indole (positive test) among the end products, whereas A. aerogenes does not (negative test).

In reviewing technical literature, the worker should be alert to the method used to detect indole formation, as the results may be greatly influenced by the analytical procedure.

4 Voges-Proskauer test (acetylmethyl carbinol test)

The test is for detection of acetylmethyl carbinol, a derivative of 2,3, butyleneglycol, as a result of glucose fermentation in the presence of peptone. A. aerogenes produces this end product (positive test) whereas E. coli gives a negative test.

a Experience with coliform cultures giving a positive test has shown a loss of this ability with storage on laboratory media for 6 months to $2\frac{1}{2}$ years, in 20 - 25% of cultures (105 out of 458 cultures).

b Some workers consider that all coliform bacteria produce acetylmethyl carbinol in glucose metabolism. These workers regard acetylmethyl carbinol-negative cultures as those which have enzyme systems capable of further degradation of acetylmethyl carbinol to other end products which do not give a positive test with the analytical procedure. Cultures giving a positive test for acetylmethyl carbinol lack this enzyme system.

c This reasoning leads to a hypothesis (not experimentally proven) that the change of reaction noted in certain cultures in 4.a above is due to the activation of a latent enzyme system.

5 Citrate utilization

Cultures of E. coli are unable to use the carbon of citrates (negative test) in their metabolism, whereas cultures of A. aerogenes are capable of using the carbon of citrates in their metabolism (positive test).

Some workers (using Simmons Citrate Agar) incorporate a pH indicator (brom thymol blue) in the culture medium in order to demonstrate the typical alkaline reaction (pH 8.4 - 9.0) resulting with citrate utilization.

6 Elevated temperature (Eijkman) test

a The test is based on evidence that E. coli and other coliforms of fecal origin are capable of growing and fermenting carbohydrates (glucose or lactose) at temperatures significantly higher than the body temperature of warm-blooded animals. Organisms not associated with direct fecal origin would give a negative test result, through their inability to grow at the elevated temperature.

b While many media and techniques have been proposed, EC Broth, a medium developed by Perry and Hajna, used as a confirmatory medium for 24 hours at 44.5 ± 0.2°C are the current recommended medium and method of choice. While the "EC" terminology of the medium suggests "*E. coli*" the worker should not regard this as a specific procedure for isolation of *E. coli.*

c A similar medium, Boric Acid Lactose Broth, has been developed by Levine and his associates. This medium gives results virtually identical with those obtained from EC Broth, but requires 48 hours of incubation.

d Elevated temperature tests require incubation in a water bath. Standard Methods 13th Ed. requires this temperature to be 44.5 ± 0.2°C. Various workers have urged use of temperatures ranging between 43.0°C and 46.0°C. Most of these recommendations have provided a tolerance of ± 0.5°C from the recommended levels. However, some workers, notably in the Shellfish Program of the Public Health Service, stipulate a temperature of 44.5 ± 0.2°C. This requires use of a water bath with forced circulation to maintain this close tolerance. This tolerance range has been instituted in the 13th Edition of Standard Methods and the laboratory worker should conform to these new limits.

e The reliability of elevated temperature tests is influenced by the time required for the newly-inoculated cultures to reach the designated incubation temperature. Critical workers insist on placement of the cultures in the water bath within 30 minutes, at most, after inoculation.

7 Other tests

Numerous other tests for differentiation between coliforms of fecal vs. nonfecal origin have been proposed. Current studies suggest little promise for the following tests in this application: uric acid test, cellobiose fermentation, gelatin liquefaction, production of hydrogen sulfide, sucrose fermentation, and others.

C IMViC Classification

1 In 1938, Parr reported on a review of a literature survey on biochemical tests used to differentiate between coliforms of fecal vs. nonfecal origin. A summary follows:

Test	No. of times used for differentiation
Voges-Proskauer reaction	22
Methyl red test	20
Citrate utilization	20
Indole test	15
Uric acid test	6
Cellobiose fermentation	4
Gelatin liquefaction	3
Eijkman test	2
Hydrogen sulfide production	1
Sucrose fermentation	1
a-Methyl-d-glucoside fermentation	1

2 Based on this summary and on his own studies, Parr recommended utilization of a combination of tests, the indole, methyl red, Voges-Proskauer, and the citrate utilization tests for this differentiation. This series of reactions is designated by the mnemonic "IMViC". Using this scheme, any coliform culture can be described by an "IMViC Code" according to the reactions for each culture. Thus, a typical culture of *E. coli* would have a code ++--, and a typical *A. aerogenes* culture would have a code --++.

3 Groupings of coliforms into fecal, non-fecal, and intermediate groups, as shown in "Standard Methods for the Examination of Water and Wastewater" are shown at the bottom of this page.

D Need for Study of Multiple Cultures

All the systems used for differentiation between coliforms of fecal vs. those of nonfecal origin require isolation and study of numerous pure cultures. Many workers prefer to study at least 100 cultures from any environmental source before attempting to categorize the probable source of the coliforms.

III NATURAL DISTRIBUTION OF COLIFORM BACTERIA

A Sources of Background Information

Details of the voluminous background of technical information on coliform bacteria recovered from one or more environmental media are beyond the scope of this discussion. References of this outline are suggested routes of entry for workers seeking to explore this topic.

B Studies on Coliform Distribution

1 Since 1960 numerous workers have engaged in a continuing study of the natural distribution of coliform bacteria and an evaluation of procedures for differentiation between coliforms of fecal vs. probable nonfecal origin. Results of this work have special significance because:

- a Rigid uniformity of laboratory methods have been applied throughout the series of studies
- b Studies are based on massive numbers of cultures, far beyond any similar studies heretofore reported

Groupings of Coliforms into Fecal, Nonfecal and Intermediate Groups

Organism	Indole	Methyl red	Voges-Proskauer	Citrate
E. coli, Variety I	+	+	-	-
Variety II	-	+	-	-
E. freundii (Intermediates)				
Variety I	-	+	-	±
Variety II	+	+	-	+
A. aerogenes				
Variety I	-	-	+	±
Variety II	±	-	+	+

c A wider variety of environmental and biological sources is being studied than in any previous series of reports.

d All studies are based on freshly recovered pure culture isolates from the designated sources.

e All studies are based on cultures recovered from the widest feasible geographic range, collected at all seasons of the year. It is believed that no more representative series of studies has been made or is in progress.

2 Distribution of coliform types

Table 1 shows the consolidated results of coliform distributions from various biological and environmental sources.

a The results of these studies show a high order of correlation between known or probable fecal origin and the typical E. coli IMViC code (++--). On the other hand, human feces also includes numbers of A. aerogenes and other IMViC types, which some regard as "nonfecal" segments of the coliform group. (Figure 1)

b The majority of coliforms attributable to excretal origin tend to be limited to a relatively small number of the possible IMViC codes; on the other hand, coliform bacteria recovered from undisturbed soil, vegetation, and insect life represent a wider range of IMViC codes than fecal sources, without clear dominance of any one type. (Figure 2)

c The most prominant IMViC code from nonfecal sources is the intermediate type, -+-+, which accounts for almost half the coliform cultures recovered from soils, and a high percentage of those recovered from vegetation and from insects. It would appear that if any coliform segment could be termed a "soil type" it would be IMViC code -+-+.

d It should not be surprising that cultures of typical E. coli are recovered in relatively smaller numbers from sources judged, on the basis of sanitary survey, to be unpolluted. There is no known way to exclude the influence of limited fecal pollution from small animals and birds in such environments.

e The distribution of coliform types from human sources should be regarded as a representative value for large numbers of sources. Investigations have shown that there can be large differences in the distribution of IMViC types from person to person, or even from an individual.

3 Differentiation between coliforms of fecal vs. nonfecal origin

Table 2 is a summary of findings based on a number of different criteria for differentiating between coliforms of fecal origin and those from other sources.

a IMViC type ++-- is a measurement of E. coli, Variety I, and appears to give reasonably good correlation between known or highly probable fecal origin and doubtful fecal origin.

b The combination of IMViC types, ++--, +---, and -+--, gives improved identification of probable fecal origin, and appears also to exclude most of the coliforms not found in excreta of warm-blooded animals in large numbers.

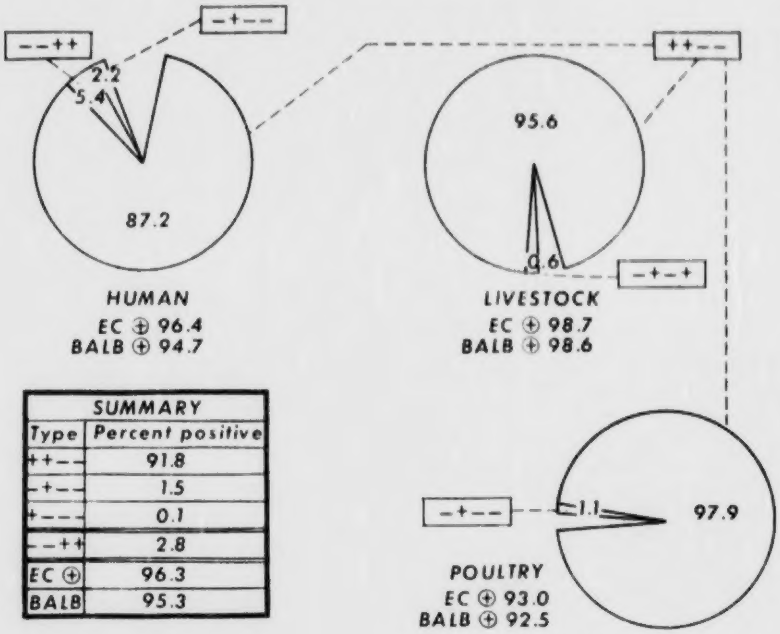
c While the indole, methyl red, Voges Proskauer, and citrate utilization tests, each used alone, appear to give useful answers when applied only to samples of known pollution from fecal sources, the interpretation is not as clear when applied to coliforms from sources believed to be remote from direct fecal pollution.

Table 1. COLIFORM DISTRIBUTION BY IMViC TYPES AND ELEVATED TEMPERATURE TEST FROM ENVIRONMENTAL AND BIOLOGICAL SOURCES

IMViC type	Vegetation		Insects		Soil				Fecal sources				Poultry	
					Undisturbed		Polluted		Human		Livestock			
	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total
++--	128	10.6	134	12.4	131	5.6	536	80.6	3932	87.2	2237	95.6	1857	97.9
--++	237	19.7	113	10.4	443	18.8	13	2.0	245	5.4	0	<0.1	1	0.1
-+--	23	1.9	0	<0.1	78	3.3	1	0.2	99	2.2	14	0.6	20	1.1
+++-	2	0.2	0	<0.1	7	0.3	0	<0.1	106	2.4	59	2.5	0	<0.1
-+-+	168	14.0	332	30.6	1131	48.1	87	13.0	50	1.1	1	<0.1	5	0.3
++-+	116	9.6	118	10.9	87	3.7	22	3.3	35	0.8	27	1.2	11	0.6
-+++	32	2.7	28	2.6	181	7.7	5	0.7	21	0.5	0	<0.1	0	<0.1
++++	291	24.2	254	23.4	159	6.8	0	<0.1	6	0.1	0	<0.1	0	<0.1
+-++	88	7.3	46	4.2	67	2.9	0	<0.1	14	0.2	0	<0.1	0	<0.1
---+	87	7.2	42	3.9	4	0.2	1	0.2	2	<0.1	0	<0.1	0	<0.1
-++-	5	0.4	0	<0.1	1	<0.1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
--+-	19	1.6	0	<0.1	53	2.3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+-+-	2	0.2	0	<0.1	6	0.3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+--+	5	0.4	8	0.7	0	<0.1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+---	0	<0.1	9	0.8	0	<0.1	0	<0.1	2	<0.1	0	<0.1	2	<0.1
Total	1203		1084		2348		665		4512		2339		1896	
No. EC +	169*		162*		216		551		4349		2309		1765	
% EC +	14.1*		14.9*		9.2		82.9		96.4		98.7		93.0	

*120 of these were ++--, 15 --++, 11 -+--

*129 of these were ++--, 27 -+-+, 5 ++-+



SUMMARY	
Type	Percent positive
++--	91.8
-+--	1.5
+---	0.1
--++	2.8
EC ⊕	96.3
BALB	95.3

FIGURE 1

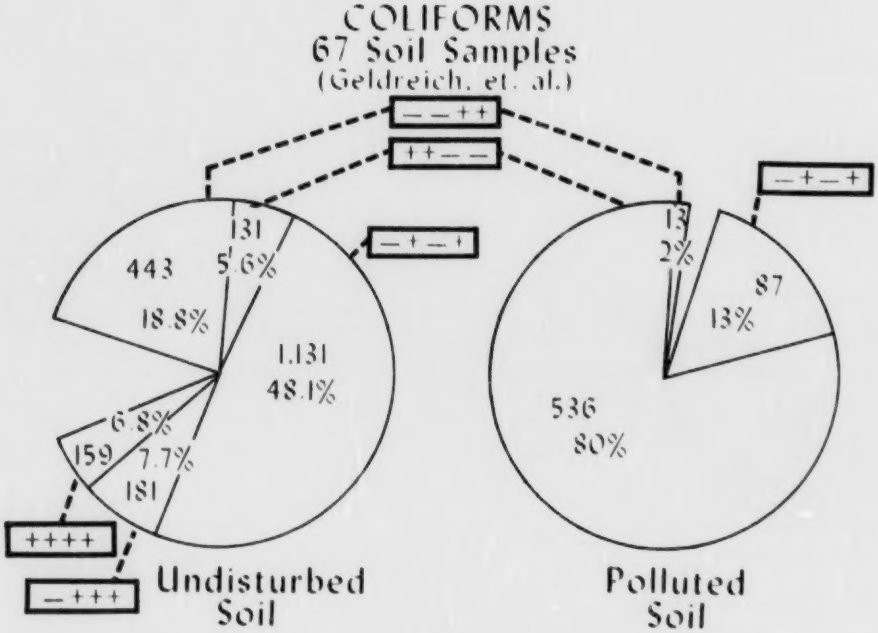


FIGURE 2

Table 2. COMPARISON OF COLIFORM STRAINS ISOLATED FROM WARM-BLOODED ANIMAL FECES, FROM UNPOLLUTED SOILS AND POLLUTED SOILS WITH USE OF THE IMViC REACTIONS AND THE ELEVATED TEMPERATURE TEST IN EC MEDIUM AT 44.5° C (±0.5°) (12th ed. 1965; Standard Methods for the Examination of Water and Wastewater)

Test	Warm-blooded animal feces	Soil: Unpolluted	Soil: Polluted	Vegetation	Insects
+ + - -	91.8%	5.6%	80.6%	10.6%	12.4%
+ + - -, + - - - and - + - -	93.3%	8.9%	80.7%	12.5%	13.2%
Indole positive	94.0%	19.4%	82.7%	52.5%	52.4%
Methyl red positive	96.9%	75.6%	97.9%	63.6%	79.9%
Voges-Proskauer positive	5.1%	40.7%	97.3%	56.3%	40.6%
Citrate utilizers	3.6%	88.2%	19.2%	85.1%	86.7%
Elevated temperature (EC) positive	96.4%	9.2%	82.9%	14.1%	14.9%
Number of cultures studied	8,747	2,348	665	1,203	1,084

Total Pure Cultures Studied: 14,047

d The elevated temperature test gives excellent correlation with samples of known or highly probable fecal origin. The presence of smaller, but demonstrable, percentages of such organisms in environmental sources not interpreted as being polluted could be attributed largely to the warm-blooded wildlife in the area, including birds, rodents, and other small mammals.

e The elevated temperature test yields results equal to those obtained from the total IMViC code. It has marked advantages in speed, ease and simplicity of performance, and yields quantitative results for each water sample. Therefore, it is regarded as the method of choice for differentiation between coliforms of probable direct fecal origin and those which may have become established in the bacterial flora of the aquatic or terrestrial habitat.

IV EVALUATION OF COLIFORMS AS POLLUTION INDICATORS

A The Coliform Group as a Whole

1 Merits

a The absence of coliform bacteria is evidence of a bacteriologically safe water.

b The density of coliforms is roughly proportional to the amount of excretal pollution present.

c If pathogenic bacteria of intestinal origin are present, coliform bacteria also are present, in much greater numbers.

d Coliforms are always present in the intestines of humans and other warm-blooded animals, and are eliminated in large numbers in fecal wastes.

e Coliforms are more persistent in the aquatic environment than are pathogenic bacteria of intestinal origin.

f Coliforms are generally harmless to humans and can be determined quantitatively by routine laboratory procedures.

2 Limitations

a Some of the constituents of the coliform group have a wide environmental distribution in addition to their occurrence in the intestines of warm-blooded animals.

b Some strains of the coliform group may multiply in certain polluted waters ("aftergrowth"), of high nutritive values thereby adding to the difficulty of evaluating a pollution situation in the aquatic environment. Members of the A. aerogenes section of the coliform are commonly involved in this kind of problem.

c Because of occasional aftergrowth problems, the age of the pollution may be difficult to evaluate under some circumstances.

d Tests for coliforms are subject to interferences due to other kinds of bacteria. False negative results sometimes occur when species of Pseudomonas are present. False positive results sometimes occur when two or more kinds of noncoliforms produce gas from lactose, when neither can do so alone (synergism).

B The Fecal Coliform Component of the Coliform Group (as determined by elevated temperature test)

1 Merits

a The majority (over 95% of the coliform bacteria from intestines of warm-blooded animals grow at the elevated temperature.

b These organisms are of relatively infrequent occurrence except in association with fecal pollution.

c Survival of the fecal coliform group is shorter in environmental waters than for the coliform group as whole. It follows, then, that high densities of fecal coliforms is indicative of relatively recent pollution.

d Fecal coliforms generally do not multiply outside the intestines of warm-blooded animals. In certain high-carbohydrate wastes, such as from the sugar beet refineries, exceptions have been noted.

2 Limitations

a Feces from warm-blooded animals include some (though proportionately low) numbers of coliforms which do not yield a positive fecal coliform test when the elevated temperature test is used as the criterion of differentiation. These organisms are E. coli varieties by present taxonomic classification.

b There is at present no established and consistent correlation between ratios of total coliforms/fecal coliforms in interpreting sanitary quality of environmental waters.

In domestic sewage, the fecal coliform density commonly is greater than 90% of the total coliform density. In environmental waters relatively free from recent pollution, the fecal coliform density may range from 10-30% of the total coliforms. There are, however, too many variables relating to water-borne wastes and surface water runoff to permit sweeping generalization on the numerical relationships between fecal- and total coliforms.

c At this time, evaluations are underway regarding the survival of fecal coliforms in polluted waters compared with that of enteric pathogenic bacteria. In recent pollution studies, species of Salmonella have been found in the presence of 220 fecal coliforms per 100 ml (Spino), and 110 fecal coliforms per 100 ml (Brezenski, Raritan Bay Project).

V APPLICATIONS OF COLIFORM TESTS

A Current Status in Official Tests

1 The coliform group is designated, in "Standard Methods for the Examination of Water and Wastewater" (13th ed., 1971), through the Completed Test MPN procedure as the official test for bacteriological potability of water. The Confirmed Test MPN procedure is accepted where it has been demonstrated, through comparative tests, to yield results equivalent to the Completed Test. The membrane filter method also is accepted for examination of waters subject to interstate regulation.

2 The 12th edition of Standard Methods introduced the standard test for fecal coliform bacteria. It is emphasized that this is to be used in pollution studies, and does not apply to the evaluation of water for potability. This procedure has been carried to the 13th Edition.

B Applications

1 Tests for the coliform group as a whole are used in official tests to comply with interstate drinking water standards, state standards for shellfish waters, and in most, if not all, cases where bacterial standards of water quality have been established for such use as in recreational or bathing waters, water supplies, or industrial supplies. Laboratory personnel should be aware of possible implementation of the fecal coliform group as the official test for recreational and bathing waters.

2 The fecal coliform test has application in water quality surveys, as an adjunct to determination of total coliform density. The fecal coliform test is being used increasingly in all water quality surveys.

3 It is emphasized that no responsible worker advocates substitution of a fecal coliform test for total coliforms in evaluating drinking water quality.

Part 3. The Fecal Streptococci

I INTRODUCTION

Investigations regarding streptococci progressed from the streptococci of medical concern to those which were distributed in differing environmental conditions which, again, related to the welfare of man. The streptococci were originally reported by Laws and Andrews (1894), and Houston (1899, 1900) considered those streptococci, which we now call "fecal streptococci," as ... "indicative of dangerous pollution, since they are readily demonstrable in waters recently polluted and seemingly altogether absent from waters above suspicion of contamination.

From their discovery to the present time the fecal streptococci appear characteristic of fecal pollution, being consistently present in both the feces of all warm-blooded animals and in the environment associated with animal discharges. As early as 1910 fecal streptococci were proposed as indicators to the Metropolitan Water Board of London. However, little progress resulted in the United States until improved methods of detection and enumeration appeared after World War II.

Renewed interest in the group as indicators began with the introduction of azide dextrose broth in 1950, (Mallmann & Seligmann, 1950). The method which is in the current edition of Standard Methods appeared soon after. (Litsky, et al. 1955).

With the advent of improved methods for detection and enumeration of fecal streptococci, significant body of technical literature has appeared.

This outline will consider the findings of various investigators regarding the fecal streptococci and the significance of discharges of these organisms into the aquatic environment.

II FECAL MATERIALS

A Definition

The terms "enterococci," "fecal streptococci," "Group D streptococci," "*Streptococcus fecalis*," and even "streptococci" have been used in a loose and interchangeable manner to indicate the streptococci present in the enteric tract of warm-blooded animals or of the fresh fecal material excreted therefrom.

Enterococci are characterized by specific taxonomic biochemistry. Serological procedures differentiate the Group D streptococci from the various groups. Although they overlap, the three groups, fecal streptococcus, enterococcus, and Group D streptococcus, are not synonymous. Because our emphasis is on indicators of unsanitary origin, fecal streptococcus is the more appropriate term and will include the enterococcus as well as other groups.

A rigid definition of the fecal streptococcus group is not possible with our present knowledge. The British Ministry of Health (1956) defines the organisms as "Gram-positive" cocci, generally occurring in pairs or short chains, growing in the presence of bile salt, usually capable of development at 45° C, producing acid but not gas in mannitol and lactose, failing to attack raffinose, failing to reduce nitrate to nitrite, producing acid in litmus milk and precipitating the casein in the form of a loose, but solid curd, and exhibiting a greater resistance to heat, to alkaline conditions and to high concentrations of salt than most vegetative bacteria." However, it is pointed out that "streptococci departing in one or more particulars from the type species cannot be disregarded in water."

For the proposes of this outline, and in line with the consensus of most water microbiologists in this country, the definition of the fecal streptococci is:

> ... "The group composed of Group D species consistently present in significant numbers in fresh fecal excreta of warm-blooded animals, which includes all of the enterococcus group in addition to other groups of streptococci."

B Species Isolated

1 Findings

a Human feces

Examination of human fecal specimens yields a high percentage of the enterococcus group and usually demonstration of the S. salivarius which is generally considered a member of the human throat flora and to be surviving in human fecal materials rather than actively multiplying in the enteric tract. Also present would be a small percentage of variants or biotypes of the enterococcus group.

b Nonhuman Feces

1) Fecal material which are from nonhuman or not from fowl will yield high percentages of the S. bovis and/or S. equinus organisms with a concomitantly reduced percentage of the enterococcus group.

2) Fowl excreta

Excrement from fowl characteristically yields a large percentage of enterococcal biotypes as well as a significant percentage of enterococcus group.

2 Significance

Species associations with particular animal hosts is an established fact and leads to the important laboratory technique of partition counting of colonies from the membrane filter or pour agar plates in order to establish or confirm the source of excretal pollution in certain aquatic investigations.

It is important to realize that a suitable medium is necessary in order to allow all of the streptococci which we consider to be fecal streptococci to grow in order to give credence to the derived opinions. Use of liquid growth media into which direct inoculations from the sample are made have not proven to be successful for partition counting due to the differing growth rates of the various species of streptococci altering the original percentage relationships. Due to the limited survival capabilities of some of the fecal streptococci it is necessary to sample fresh fecal material or water samples in close proximity to the pollution source especially when multiple sources are contributing to a reach of water. Also the pH range must be within the range of 4.0-9.0.

III FECAL STREPTOCOCCI IN THE AQUATIC ENVIRONMENT

A General

From the foregoing it is apparent that the preponderant human fecal streptococci is composed of the enterococcus group and, as this is the case, several media presently available which will detect only the enterococcal group will be suitable for use with aquatic samples which are known to be contaminated or potentially contaminated with purely domestic (human) wastes. On the other hand, when it is known or suspected that other-than-human wastes have potential egress to the aquatic environment under investigation, it is necessary to utilize those media which are capable of quantitating the whole of the fecal streptococci group.

B Stormwaters and Combined Sewers

1 General

Storm sewers are a series of pipes and conduits which receive surface runoffs from the action of rainstorms and do not include sewage which are borne by a system of sanitary sewers. Combined sewers receive both the runoff rains as well as the water borne wastes of the sanitary system. Both of these types of runoffs can be discharged to the aquatic environment and the usual instance where this occurs, with respect to the combined sewers, is when the amount of flow is in excess of the amounts capable of being treated during the high flow conditions. Both of these discharge forms have been found to usually contain large quantities of fecal streptococci and in numbers which generally are larger than that of the fecal coliform indicator organism. Stormwaters can be concluded to represent a typical stream environment with respect to the presence of chemical constituents and show a wide range of electrolytes which at times simulate that of irrigation waters.

2 Bacteriological Findings

Table 1 represents, in a modified form, some of the findings of Geldreich and Kenner (1969) with respect to the densities of fecal streptococci when considering Domestic sewage in contrast to Stormwaters:

Table 1

DISTRIBUTION OF FECAL STREPTOCOCCI IN DOMESTIC SEWAGES AND STORMWATER RUNOFFS

Water Source	Fecal Streptococci per 100 ml median values	Ratio FC/FS
Domestic Sewage		
Preston, ID	64,000	5.3
Fargo, ND	290,000	4.5
Moorehead, MN	330,000	4.9
Cincinnati, OH	2,470,000	4.4
Lawrence, MA	4,500,000	4.0
Monroe, MI	700,000	27.9
Denver, CO	2,900,000	16.9
Stormwater		
Business District	51,000	0.26
Residential	150,000	0.04
Rural	58,000	0.05

The Ratio FC/FS is that of the Fecal coliform and Fecal streptococci and it will be noted that in each case, when considering the Domestic Sewage, it is 4.0 or greater while it is less than 0.7 for stormwaters. The use of this ratio is useful to identify the source of pollution as

Table 2. ESTIMATED PER CAPITA CONTRIBUTION OF INDICATOR MICROORGANISMS FROM SOME ANIMALS*

		Average indicator density per gram of feces		Average contribution per capita per 24 hr		
Animals	Avg wt of Feces/24 hr, wet wt, g	Fecal coliform, million	Fecal streptococci, million	Fecal coliform, million	Fecal streptococci, million	Ratio FC/FS
Man	150	13.0	3.0	2,000	450	4.4
Duck	336	33.0	54.0	11,000	18,000	0.6
Sheep	1,130	16.0	38.0	18,000	43,000	0.4
Chicken	182	1.3	3.4	240	620	0.4
Cow	23,600	0.23	1.3	5,400	31,000	0.2
Turkey	448	0.29	2.8	130	1,300	0.1
Pig	2,700	3.3	84.0	8,900	230,000	0.04

*Publication WP-20-3, P. 102

being human or nonhuman warm-blooded animal polluted. When the ratio is greater than 4.0 it is considered to be human waste contaminated while a ratio of less than 0.7 is considered to be nonhuman. It is evident that the storm-waters have been primarily polluted by excreta of rats and other rodents and possibly domestic and/or farm animals.

Species differences are the main cause of different fecal coliform-fecal streptococci ratios. Table 2 compares fecal streptococcus and fecal coliform counts for different species. Even though individuals vary widely, masses of individuals in a species have characteristic proportion of indicators.

C Surface Waters

In general, the occurrence of fecal streptococci indicates fecal pollution and its absence indicates that little or no warm-blooded fecal contribution. In studies of remote surface waters the fecal streptococci are infrequently isolated and occurrences of small numbers can be attributed to wild life and/or snow melts and resultant drainage flows.

Various examples of fecal streptococcal occurrences are shown in Table 3 in relation to surface waters of widely varying quality. (Geldreich and Kenner 1969)

Table 3

INDICATOR ORGANISMS IN SURFACE WATERS

	Densities/100 ml	
Water Source	Fecal coliform	Fecal streptococci
Prairie Watersheds		
Cherry Creek, WY	90	83
Saline River, KS	95	180
Cub River, ID	110	160
Clear Creek, CO	170	110
Recreational Waters		
Lake Mead	2	444
Lake Moovalaya	9	170
Colorado River	4	256
Whitman River	32	88
Merrimack River	100	96
Public Water Intakes		
Missouri River (1959)		
Mile 470.5	11,500	39,500
Mile 434.5	22,000	79,000
Mile 408.8	14,000	59,000

IV FECAL STREPTOCOCCI: ADVANTAGES AND LIMITATIONS

A General

Serious studies concerning the streptococci were instituted when it became apparent that they were the agents responsible or suspected for a wide variety of human diseases. Natural priority then focused itself to the taxonomy of these organisms and this study is still causing consternation as more and more microbiological techniques have been brought to bear on these questions. The sanitary microbiologist is concerned with those streptococci which inhabit the enteric tract of warm-blooded animals, their detection, and utilization in developing a criterium for water quality standards.

Kabler (1962) discussed the slow acceptance of the fecal streptococci as indicators of pollution resulting from:

1 Multiplicity and difficulty of laboratory procedures

2 Poor agreement between methods of quantitative enumeration

3 Lack of systematic studies of . . . .

  a sources

  b survival, and

  c interpretations, and

4 Undue attention to the S. faecalis group.

Increased attention to the fecal streptococci, especially during the last decade, have clarified many of the earlier cloudy issues and have elevated the stature of these organisms as indicators of pollution. Court precedents establishing legal status and recommendations of various technical advisory boards have placed the fecal coliform group in a position of primacy in many water quality applications. The fecal streptococci have evolved from a position of a theoretically useful indicator to one which was ancillary to the coliforms to one which was useful when discrepancies or questions evolved as to the validity of the coliform data to one where an equality status was achieved in certain applications. In the future it is anticipated that, for certain applications, the fecal streptococci will achieve a position of primacy for useful data, and, as indicated by Litsky (1955) "be taken out of the realm of step-children and given their legitimate place in the field of santiary bacteriology as indicators of sewage pollution."

B Advantages and Limitations

1 Survival

In general, the fecal streptococci have been observed to have a more limited survival time in the aquatic environment when compared to the coliform group. They are rivaled in this respect only by the fecal coliforms. Except for cases of persistence in waters of high electrolytic content, as may be common to irrigation waters, the fecal streptococci have not been observed to multiply in polluted waters as may sometimes be observed for some of the coliforms. Fecal streptococci usually require a greater abundance of nutrients for survival as compared to the coliforms and the coliforms are more dependent upon the oxygen tension in the waterbody. In a number of situations it was concluded that the fecal streptococci reached an extinction point more rapidly in warmer waters while the reverse was true in the colder situations as the coliforms now were totally eliminated sooner.

2 Resistance to Disinfection

In artificial pools the source of contamination by the bathers is usually limited to throat and skin flora and thus increasing attention has been paid to indicators other than those traditionally from the enteric tract. Thus, one of the organisms considered to be a fecal streptococci, namely, S. salivarius, can be a more reliable indicator when detected along with the other fecal streptococci especially since studies have confirmed the greater resistance of the fecal streptococci to chlorination. This greater resistance to chlorination, when compared to the fecal coliforms, is important since the dieoff curve differences are insignificant when the curves of the fecal coliforms are compared to various Gram negative pathogenic bacteria which reduces their effectiveness as indicators.

3 Ubiquitous Strains

Among the fecal streptococcus are two organisms, one a biotype and the other a variety of the S. faecalis, which, being ubiquitous (omnipresent) have limited sanitary significance. The biotype, or atypical, S. faecalis is characterized by its ability to hydrolyze starch while the varietal form, liquefaciens, is nonbeta haemolytic and capable of liquefying gelatin. Quantitation of these organisms in anomalous conditions is due to their capability of survival in soil or high electrolytic waters and in waters with a temperature of less than 12 Degrees C.

Samples have been encountered which have been devoid of fecal coliforms and yet contain a substantial number of "fecal streptococci" of which these ubiquitous strains constitute the majority or all of the isolations when analyzed biochemically.

V STANDARDS AND CRITERIA

Acceptance and utilization of Total Coliform criteria, which must now be considered a pioneering effort, has largely been supplanted in concept and in fact by the fecal coliforms in establishing standards for recreational waters.

The first significant approach to the utilization of the fecal streptococci as a criterium for recreational water standards occurred in 1966 when a technical committee recommended the utilization of the fecal streptococci with the total coliforms as criteria for standards pertaining to the Calumet River and lower Lake Michigan waters. Several sets of criteria were established to fit the intended uses for this area. The use of the fecal streptococci as a criterium is indicated to be tentative pending the accumulation of existing densities and could be modified in future standards.

With the existing state-of-the-art knowledge of the presence of the fecal streptococci in waters containing low numbers of fecal coliforms it is difficult to establish a specific fecal streptococcus density limit of below 100 organisms/100 ml when used alone or in conjunction with the total coliforms.

Part 4. Other Bacterial Indicators of Pollution

I TOTAL BACTERIAL COUNTS

A Historical

1 The early studies of Robert Koch led him to develop tentative standards of water quality based on a limitation of not more than 100 bacterial colonies per ml on a gelatin plating medium incubated 3 days at 20° C.

2 Later developments led to inoculation of samples on duplicate plating media, with one set incubated at 37° C and the other at 20° C.

a Results were used to develop a ratio between the 37° C counts and the 20° C counts.

b Waters having a predominant count at 37° C were regarded as being of probable sanitary significance, while those giving predominant counts at 20° C were considered to be of probable soil origin, or natural inhabitants of the water being examined.

B Groups Tested

There is no such thing as "total" bacterial count in terms of a laboratory determination.

1 Direct microscopic counts do not differentiate between living and dead cells.

2 Plate counting methods enumerate only the bacteria which are capable of using the culture medium provided, under the temperature and other growth conditions used as a standard procedure. No one culture medium and set of growth conditions can provide, simultaneously, an acceptable environment for all the heterogeneous, often conflicting, requirements of the total range of bacteria which may be recovered from waters.

C Utilization of Total Counts

1 Total bacterial counts, using plating methods, are useful for:

a Detection of changes in the bacterial composition of a water source

b Process control procedures in treatment plant operations

c Determination of sanitary conditions in plant equipment or distributional systems

2 Serious limitations in total bacterial counts exist because:

a No information is given regarding possible or probable fecal origin of bacterial changes. Large numbers of bacteria can sometimes be cultivated from waters known to be free of fecal pollution.

b No information of any kind is given about the species of bacteria cultivated.

c There is no differentiation between harmless or potentially dangerous forms.

3 Status of total counts

a There is no total bacterial count standard for any of the following:

Interstate Quarantine Drinking Water Standards

PHS regulations for water potability (as shown in "Standard Methods" Public Health Service Drinking Water Standards of 1962.)

b The most widely used current application of total bacterial counts in water bacteriology today is in water treatment plants, where some workers use standard plate counts for process control and for determination of the bacterial quality of distribution systems and equipment.

c Total bacterial counts are not used in PHS water quality studies, though extensively used until the 1940's.

B Spore-Forming Bacteria (*Clostridium perfringens*, or *C. welchii*)

1 Distribution

This is one of the most widely distributed species of bacteria. It is regularly present in the intestinal tract of warm-blooded animals.

2 Nature of organism

C. perfringens is a Gram-positive, spore-forming rod. The spores cause a distinct swelling of the cell when formed. The organism is extremely active in fermentation of carbohydrates, and produces the well-known "stormy fermentation" of milk.

3 Status

The organism, when present, indicates that pollution has occurred at some time. However, because of the extremely extended viability of the spores, it is impossible to obtain even an approximation of the recency of pollution based only on the presence of *C. perfringens*.

The presence of the organism does not necessarily indicate an unsafe water.

C Tests for Pathogenic Bacteria of Intestinal Origin

1 Groups considered include *Salmonella* sp, *Shigella* sp, *Vibrio comma*, *Mycobacterium* sp, *Pasteurella* sp, *Leptospira* sp, and others.

2 Merits of direct tests:

Demonstration of any pathogenic species would demonstrate an unsatisfactory water quality, hazardous to persons consuming or coming into contact with that water.

3 Limitations

a There is no available routine procedure for detection of the full range of pathogenic bacteria cited above.

b Quantitative methods are not available for routine application to any of the above.

c The intermittent release of these pathogens makes it impossible to regard water as safe, even in the absence of pathogens.

d After detection, the public already would have been exposed to the organism; thus, there is no built-in margin of safety, as exists with tests for the coliform group.

4 Applications

a In tracing the source of pathogenic bacteria in epidemiological investigations

b In special research projects

c In water quality studies concerned with enforcement actions against pollution, increasing attention is being given to the demonstration of enteric pathogenic bacteria in the presence of the bacterial indicators of pollution.

D Miscellaneous Indicators

It is beyond this discussion to explore the total range of microbiological indicators of pollution that have been proposed and investigated to some extent. Mention can be made, however, of consideration of tests for the following.

1 Bacteriophages specific for any of a number of kinds of bacteria

2 Serological procedures for detection of coliforms and other indicators; a certain amount of recent attention has been given to applications of fluorescent antibodies in such tests

3 Tests for *Pseudomonas aeruginosa*

4 Tests for viruses, which may persist in waters even longer than members of the coliform group.

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Descriptors: Coliforms, Escherichia coli, Fecal Coliforms, Fecal Streptococci, Indicator Bacteria, Microbiology, Sewage Bacteria, Water Pollution

EXAMINATION OF WATER FOR COLIFORM AND FECAL STREPTOCOCCUS GROUPS
(Multiple Dilution Tube (MPN) Methods)

I INTRODUCTION

The subject matter of this outline is contained in three parts, as follows:

A Part 1

1 Fundamental aspects of multiple dilution tube ("most probable numbers") tests, both from a qualitative and a quantitative viewpoint.

2 Laboratory bench records.

3 Useful techniques in multiple dilution tube methods.

4 Standard supplies, equipment, and media in multiple dilution tube tests.

B Part 2

Detailed, day-by-day, procedures in tests for the coliform group and subgroups within the coliform group.

C Part 3

Detailed, day-by-day, procedures in tests for members of the fecal streptococci.

D Application of Tests to Routine Examinations

The following considerations (Table 1) apply to the selection of the Presumptive Test, the Confirmed Test, and the Completed Test. Termination of testing at the Presumptive Test level is not practiced by laboratories of this agency. It must be realized that the Presumptive Test alone has limited use when water quality is to be determined.

TABLE 1

	Examination Terminated at -		
Type of Receiving Water	Presumptive Test	Confirmed Test	Completed Test
Sewage Receiving	Applicable	Applicable	Important where results are to be used for control of raw or finished water. Application to a statistically valid number of samples from the Confirmed Test to establish its validity in determining the sanitary quality.
Treatment Plant - Raw	Applicable	Applicable	
Chlorinated	Not Done	Applicable	
Bathing	Not Done	Applicable	
Drinking	Not Done	Applicable	
Other Information		Applicable in all cases where Presumptive Test alone is unreliable.	

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Environmental Protection Agency.

II BASIS OF MULTIPLE TUBE TESTS

A Qualitative Aspects

1 For purely qualitative aspects of testing for indicator organisms, it is convenient to consider the tests applied to one sample portion, inoculated into a tube of culture medium, and the follow-up examinations and tests on results of the original inoculation. Results of testing procedures are definite: positive (presence of the organism-group is demonstrated) or negative (presence of the organism-group is not demonstrated.)

2 Test procedures are based on certain fundamental assumptions:

a First, even if only one living cell of the test organism is present in the sample, it will be able to grow when introduced into the primary inoculation medium;

b Second, growth of the test organism in the culture medium will produce a result which indicates presence of the test organism; and,

c Third, extraneous organisms will not grow, or if they do grow, they will not limit growth of the test organism; nor will they produce growth effects that will be confused with those of the bacterial group for which the test is designed.

3 Meeting these assumptions usually makes it necessary to conduct the tests in a series of stages (for example, the Presumptive, Confirmed, and Completed Test stages, respectively, of standard tests for the coliform group).

4 Features of a full, multi-stage test

a First stage: The culture medium usually serves primarily as an enrichment medium for the group tested. A good first-stage growth medium should support growth of all the living cells of the group tested, and it should include provision for indicating the presence of the test organism being studied. A first-stage medium may include some component which inhibits growth of extraneous bacteria, but this feature never should be included if it also inhibits growth of any cells of the group for which the test is designed. The Presumptive Test for the coliform group is a good example. The medium supports growth, presumably, of all living cells of the coliform group; the culture container has a fermentation vial for demonstration of gas production resulting from lactose fermentation by coliform bacteria, if present; and sodium lauryl sulfate may be included in one of the approved media for suppression of growth of certain noncoliform bacteria. This additive apparently has no adverse effect on growth of members of the coliform group in the concentration used. If the result of the first-stage test is negative, the study of the culture is terminated, and the result is recorded as a negative test. No further study is made of negative tests. If the result of the first-stage test is positive, the culture may be subjected to further study to verify the findings of the first stage.

b Second stage: A transfer is made from positive cultures of the first-stage test to a second culture medium. This test stage emphasizes provision to reduce confusion of results due to growth effects of extraneous bacteria, commonly achieved by addition of selective inhibitory agents. (The Confirmed Test for coliforms meets these requirements. Lactose and fermentation vials are provided for demonstration of coliforms in the medium. Brilliant green dye and bile salts are included as inhibitory agents which tend to suppress growth of practically all kinds of noncoliform bacteria, but do not suppress growth of coliform bacteria when used as directed).

If result of the second-stange test is negative, the study of the culture is terminated, and the result is recorded as a negative test. A negative test here means that the positive results of the first-stage test were "false positive," due to one or more kinds of extraneous bacteria. A positive second-stage test is partial confirmation of the positive results obtained in the first-stage test; the culture may be subjected to final identification through application of still further testing procedures. In routine practice, most sample examinations are terminated at the end of the second stage, on the assumption that the result would be positive if carried to the third, and final stage. This practice should be followed only if adequate testing is done to demonstrate that the assumption is valid. Some workers recommend continuing at least 5% of all sample examinations to the third stage to demonstrate the reliability of the second-stage results.

B Quantitative Aspects of Tests

1 These methods for determining bacterial numbers are based on the assumption that the bacteria can be separated from one another (by shaking or other means) resulting in a suspension of individual bacterial cells, uniformly distributed through the original sample when the primary inoculation is made.

2 Multiple dilution tube tests for quantitative determinations apply a Most Probable Number (MPN) technique. In this procedure one or more measured portions of each of a stipulated series of decreasing sample volumes is inoculated into the first-stage culture medium. Through decreasing the sample increments, eventually a volume is reached where only one cell is introduced into some tubes, and no cells are introduced into other tubes. Each of the several tubes of sample-inoculated first-stage medium is tested independently, according to the principles previously described, in the qualitative aspects of testing procedures.

3 The combination of positive and negative results is used in an application of probability mathematics to secure a single MPN value for the sample.

4 To obtain MPN values, the following conditions must be met:

a The testing procedure must result in one or more tubes in which the test organism is demonstrated to be present; and

b The testing procedure must result in one or more tubes in which the test organism is not demonstrated to be present.

5 The MPN value for a given sample is obtained through the use of MPN Tables. It is emphasized that the precision of an individual MPN value is not great when compared with most physical or chemical determinations.

6 Standard practice in water pollution surveys conducted by this organization, is to plant five tubes in each of a series of sample increments, in sample volumes decreasing at decimal intervals. For example, in testing known polluted waters, the initial sample inoculations might consist of 5 tubes each in volumes of 0.1, 0.01, 0.001, and 0.0001 ml, respectively. This series of sample volumes will yield determinate results from a low of 200 to a high of 1,600,000 organisms per 100 ml.

III LABORATORY BENCH RECORDS

A Features of a Good Bench Record Sheet

1 Provides complete identification of the sample.

2 Provides for full, day-by-day information about all tests performed on the sample.

3 Provides easy step-by-step record applicable to any portion of the sample.

4 Provides for recording of the quantitative result which will be transcribed to subsequent reports.

5 Minimizes the amount of writing by the analyst.

6 Identifies the analyst(s).

B There is no such thing as "standard" bench sheet for multiple tube tests; there are many versions of bench sheets. Some are prescribed by administrative authority (such as the Office of a State Sanitary Engineer); others are devised by laboratory or project personnel to meet specific needs.

It is not the purpose of this discussion to recommend an "ideal" bench form; however, the form used in this training course manual is essentially similar to that used in certain research laboratories of this organization. The student enrolled in the course for which this manual is written should make himself thoroughly familiar with the bench sheet and its proper use. See Figure 1.

IV NOTES ABOUT WORKING PROCEDURES IN THE LABORATORY

A Each bacteriological examination of water by multiple dilution tube methods requires a considerable amount of manipulation; much is quite repetitious. Laboratory workers must develop and maintain good routine working habits, with constant alertness to guard against lapses into careless, slip-shod laboratory procedures and "short cuts" which only can lead to lowered quality of laboratory work.

The student reader is urged to review the form for laboratory surveys (PHS-875, Rev. 1966) used by Public Health Service personnel charged with responsibility for accreditation of laboratories for examination of water under Interstate Quarantine regulations.

B Specific attention is brought to the following by no means exhaustive, critical aspects of laboratory procedures in multiple dilution tube tests:

1 Original sample

a Follow prescribed care and handling procedures before testing.

b Maintain absolute identification of sample at all stages in testing.

c Vigorously shake samples (and sample dilutions) before planting in culture media.

2 Sample measurement into primary culture medium

a Sample portions must be measured accurately into the culture medium for reliable quantitative tests to be made. Standard Methods prescribes that calibration errors should not exceed ± 2.5%.

BACTERIOLOGY BENCH SHEET Multiple Dilution Tube Tests

Project Ohio River Survey
Sample Station Broadway Landing

Collection Data

Date 2/6/67 Time 8:50 By KJ
Temperature 8 °C pH 7.3
Other Observations

Analytical Record

Bench Number of Sample 2
Analyst Zobel-Jeter
Test started at 11:45 By HLJ

ml mple	Coliform Test								Fecal coli-form	Fecal Streptococcus				Remarks
	LTB		BGLB		EMB	LSTB		Gram stain		A - D		EVA		
	24	48	24	48	24	24	48		24	24	48	24	48	
10														
1.0														
0.1														
0.01														
0.001														
0.001														

Coliform MPN/100 ml
Confirmed:
Completed:
Fecal Coliform MPN:

Fecal Streptococcus MPN/100 ml
A - D - EVA:

Figure 1. SAMPLE BENCH SHEET

Suggested sample measuring practices are as follows: Mohr measuring pipets are recommended. 10 ml samples are delivered at the top of the culture tube, using 10 ml pipets. 1.0 ml samples are delivered down into the culture tube, near the surface of the medium, and "touched off" at the side of the tube when the desired amount of sample has been delivered. 1.0 ml or 2.0 ml pipets are used for measurement of this volume. 0.1 ml samples are delivered in the same manner as 1.0 ml samples, using great care that the sample actually gets into the culture medium. Only 1.0 ml pipets are used for this sample volume. After delivery of all sample increments into the culture tubes, the entire rack of culture tubes may be shaken gently to carry down any of the sample adhering to the wall of the tube above the medium.

Workers should demonstrate by actual tests that the pipets and the technique in use actually delivers the rated volumes within the prescribed limits of error.

b Volumes as small as 0.1 ml routinely can be delivered directly from the sample with suitable pipets. Lesser sample volumes first should be diluted, with subsequent delivery of suitable volumes of diluted sample into the culture medium. A diagrammatic scheme for making dilutions is shown in Figure 2.

3 Reading of culture tubes for gas production

a On removal from the incubator, shake culture rack gently, to encourage release of gas which may be supersaturated in the culture medium. .

b Gas in any quantity is a positive test. It is necessary to work in conditions of suitable lighting for easy recognition of the extremely small amounts of gas inside the tops of some fermentation vials.

4 Reading of liquid culture tubes for growth as indication of a positive test requires good lighting. Growth is shown by any amount of increased turbidity or opalescence in the culture medium, with or without deposit of sediment at the bottom of the tube.

5 Transfer of cultures with inoculation loops and needles

a Always sterilize inoculation loops and needles in flame immediately before transfer of culture; do not lay it down or touch it to any nonsterile object before making the transfer.

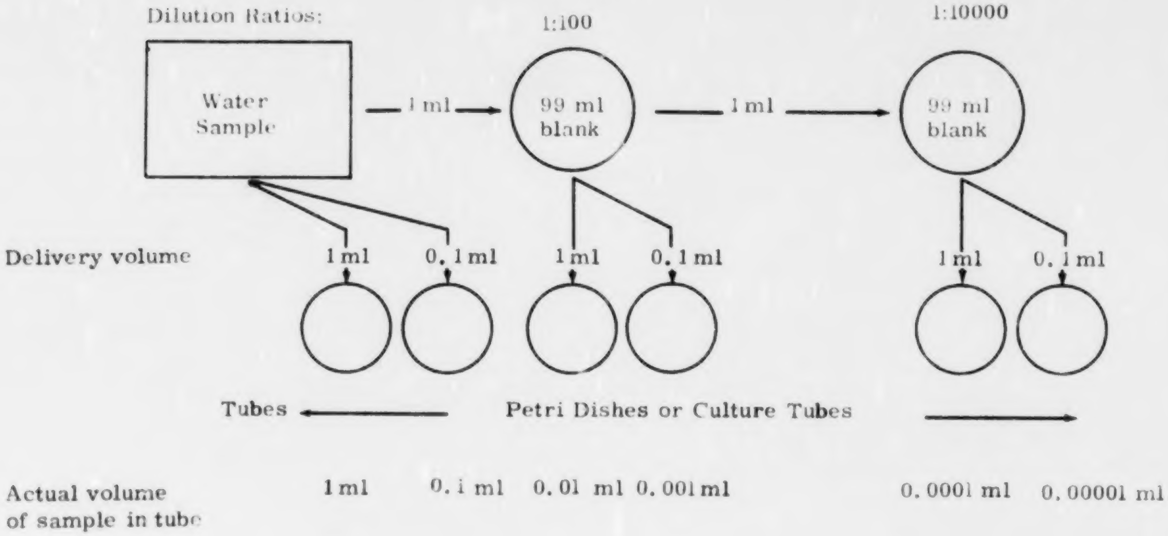
b After sterilization, allow sufficient time for cooling, in the air, to avoid heat-killing bacterial cells on the hot wire.

c Loops should be 3 mm in inside diameter, with a capability of holding a drop of water or culture.

For routine standard transfers requiring transfer of 3 loopsful of culture, many workers form three 3-mm loops on the same length of wire.

6 As an alternative to use of standard inoculation loops, the use of "applicator sticks" have now been sanctioned by the 13th Edition of Standard Methods.

Figure 2. PREPARATION OF DILUTIONS


Dilution Ratios:
1:100
1:10000
Water Sample
1 ml
99 ml blank
1 ml
99 ml blank
Delivery volume
1 ml
0.1 ml
1 ml
0.1 ml
1 ml
0.1 ml
Tubes
Petri Dishes or Culture Tubes
Actual volume of sample in tube
1 ml
0.1 ml
0.01 ml
0.001 ml
0.0001 ml
0.00001 ml

The applicator sticks are dry heat sterilized (autoclave sterilization is not acceptable because of possible release of phenols if the wood is steamed) and are used on a single-service basis. Thus, for every culture tube transferred, a new applicator stick is used.

This use of applicator sticks is particularly attractive in field situations where it is inconvenient or impossible to provide a gas burner suitable for sterilization of the inoculation loop. In addition, use of applicator sticks is favored in laboratories where room temperatures are significantly elevated by use of gas burners.

7 Streaking cultures on agar surfaces

a All streak-inoculations should be made without breaking the surface of the agar. Learn to use a light touch with the needle; however, many inoculation needles are so sharp that they are virtually useless in this respect. When the needle is platinum or platinum-iridium wire, it sometimes is beneficial to fuse the working tip into a small sphere. This can be done by momentary insertion of a well-insulated (against electricity) wire into a carbon arc, or some other extremely hot environment. The sphere should not be more than twice the diameter of the wire from which it is formed, otherwise it will be entirely too heat-retentive to be useful.

When the needle is nichrome resistance wire, it cannot be heat-fused; the writer prefers to bend the terminal 1/16 - 1/8" of the wire at a slight angle to the overall axis of the needle. The side of the terminal bent portion of the needle then is used for inoculation of agar surfaces.

b When streaking for colony isolation, avoid using too much inoculum. The streaking pattern is somewhat variable according to individual preference. The procedure favored by the writer is shown in the accompanying figures. Note particularly that when going from any one stage of the streaking to the next, the inoculation needle is heat-sterilized.

8 Preparation of cultures for Gram stain

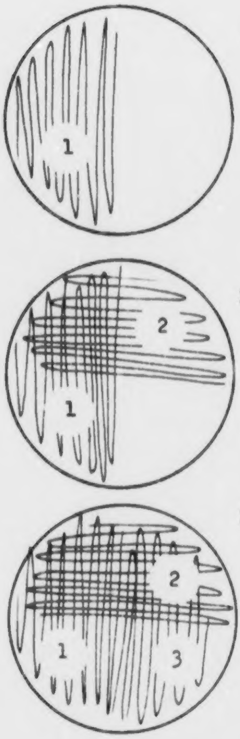
a The Gram stain always should be made from a culture grown on a nutrient agar surface (nutrient agar slants are used here) or from nutrient broth.

b The culture should be young, and should be actively growing. Many workers doubt the validity of the Gram stain made on a culture more than 24 hours old.

c Prepare a thin smear for the staining procedure. Most beginning workers tend to use too much bacterial suspension in preparing the dried smear for staining. The amount of bacteria should be so small that the dried film is barely visible to the naked eye.

V EQUIPMENT AND SUPPLIES

Consolidated lists of equipment, supplies, and culture media required for all multiple dilution tube tests described in this outline are shown in Table 2. Quantitative information is not presented; this is variable according to the extent of the testing procedure, the number of dilutions used, and the number of replicate tubes per dilution. It is noted that requirements for alternate procedures are fully listed and choices are made in accordance to laboratory preference.



1 a Flame-sterilize an inoculation needle and air-cool.

b Dip the tip of the inoculation needle into the bacterial culture being studied.

c Streak the inoculation needle tip lightly back and forth over half the agar surface, as in (1), avoiding scratching or breaking the agar surface.

d Flame-sterilize the inoculation needle and air-cool.

2 a Turn the Petri dish one-quarter-turn and streak the inoculation needle tip lightly back and forth over one-half the agar surface, working from area (1) into one-half the unstreaked area of the agar.

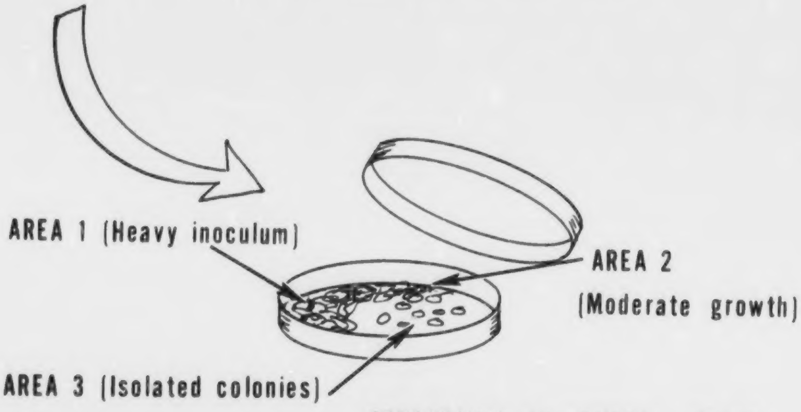
b Flame-sterilize the inoculation needle and air-cool.

3 a Turn the Petri dish one-quarter-turn and streak the inoculation needle tip lightly back and forth over one-half the agar surface, working from area (2) into area (3), the remaining unstreaked area.

b Flame-sterilize the inoculation needle and set it aside.

c Close the culture container and incubate as prescribed.

Figure 3. A SUGGESTED PROCEDURE FOR COLONY ISOLATION BY A STREAK-PLATE TECHNIQUE



APPEARANCE OF STREAK - PLATE AFTER INCUBATION INTERVAL

TABLE 2. APPARATUS AND SUPPLIES FOR STANDARD FERMENTATION TUBE TESTS

Description of Item	Total Coliform Group			Fecal Coliform	
	Presumptive Test	Confirmed Test	Completed Test	(BALB)	(EC broth
Lauryl tryptose broth or Lactose broth. 20 ml amounts of 1.5 × concentration medium, in 25 × 150 mm culture tubes with inverted fermentation vials, suitable caps.	X				
Lauryl tryptose broth or Lactose broth. 10 ml amounts of single strength medium in 20 × 150 mm culture tubes with inverted fermentation vials, suitable caps.	X		X		
Brilliant green lactose bile broth, 2% in 10 ml amounts, single strength, in 20 × 150 mm culture tubes with inverted fermentation vials, suitable caps.		X	X		
Eosin methylene blue agar, poured in 100 × 15 mm Petri dishes		X	X		
Endo Agar, poured in 100 × 15 mm dishes		X			
Nutrient agar slant, screw cap tube			X		
Boric acid lactose broth, 10 ml amounts of single strength medium in fermentation tubes.				X	
EC Broth, 10 ml amounts of single strength medium in fermentation tubes.					X
Formate ricinoleate broth (provisional)			X		
Culture tube racks, 10 × 5 openings, each opening to accept 25 mm diameter tubes.	X	X	X	X	X
Pipettes, 10 ml, Mohr type, sterile, in suitable cans.	X				
Pipettes, 2 ml (optional), Morh type, sterile, in suitable cans	X				
Pipettes, 1 ml, Mohr type, sterile in metal suitable cans	X				
Standard buffered dilution water, sterile, 99-ml amounts in screw-capped bottles.	X				
Gas burner, Bunsen type		X	X	X	X
Inoculation loop, loop 3mm diameter, of nichrome or platinum-iridium wire, 26 B & S gauge, in suitable holder. (or sterile applicator stick)		X	X	X	X
Inoculation needle, nichrome, or platinum-iridium wire, 26 B & S gauge, in suitable holder.		X	X		
Incubator, adjusted to 35 ± 0.5°C	X	X	X		
Waterbath incubator, adjusted to 43 ± 0.2°C				X	
Waterbath incubator, adjusted to 44.5 ± 0.2°C.					X
Glass microscopic slides, 1" × 3"			X		
Slide racks (optional)			X		
Gram-stain solutions, complete set			X		
Compound microscope, oil immersion lens, Abbe' condenser			X		
Basket for discarded cultures	X	X	X	X	X
Container for discarded pipettes	X				

Part 2

DETAILED TESTING PROCEDURES FOR MEMBERS OF THE COLIFORM GROUP BY MULTIPLE DILUTION TUBE METHODS

I SCOPE

A Tests Described

1 Presumptive Test

2 Confirmed Test

3 Completed Test

4 Fecal Coliform Test

B Form of Presentation

The Presumptive, Confirmed, and Completed Tests are presented as total, independent procedures. It is recognized that this form of presentation is somewhat repetitious, inasmuch as the Presumptive Test is preliminary to the Confirmed Test, and both the Presumptive Test and the Confirmed Test are preliminary to the Completed Test for total coliforms.

In using these procedures, the worker must know at the outset what is to be the stage at which the test is to be ended, and the details of the procedures throughout, in order to prevent the possibility of discarding gas-positive tubes before proper transfer procedures have been followed.

Thus, if the worker knows that the test will be ended at the Confirmed Test, he will turn at once to Section III, TESTING TO THE CONFIRMED TEST STAGE, and will ignore Sections II and IV.

The Fecal Coliform Test is described separately, in Section V, as an adjunct to the Confirmed Test and to the Completed Test.

II TESTING TO PRESUMPTIVE TEST STAGE

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory, name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample (Table 3). The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated.

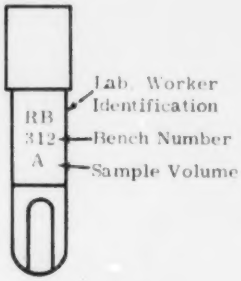
NOTE: Be sure to use tubes containing the correct concentrations of culture medium for the inoculum/tube volumes. (See the chapter on media and solutions for multiple dilution tube methods or refer to the current edition of Standard Methods for Water and Wastewater).

Table 3. SUGGESTED LABELING SCHEME FOR ORIGINAL CULTURES AND SUBCULTURES IN MULTIPLE DILUTION TUBE TESTS

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Sample volume represented
Bench number Volume & tube	312 A	312 B	312 C	312 D	312 E	Tubes with 10 ml of sample
Bench number Volume & tube	312 a	312 b	312 c	312 d	312 e	Tubes with 1 ml of sample
Bench number Volume & tube	312 a	312 b	312 c	312 d	312 e	Tubes with 0.1 ml of sample
Bench number Volume & tube	312 1a	312 1b	312 1c	312 1d	312 1e	Tubes with 0.01 ml of sample
Bench number Volume & tube	312 2a	312 2b	312 2c	312 2d	312 2e	Tubes with 0.001 ml of sample

The labeling of cultures can be reduced by labeling only the first tube of each series of identical sample volumes in the initial planting of the sample. All subcultures from initial plantings should be labeled completely.

Typical Example



Tube of Culture Medium

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an arc of one foot within seven seconds and withdraw the sample portion at once.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

  a Use a 10 ml pipet for 10 ml sample portions, and 1 ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not remove the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

  b When using the pipet to withdraw sample portions, do not dip the pipet more than 1/2 inch into the sample; otherwise sample running down the outside of the pipet will make measurements inaccurate.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5°C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Discard all gas-positive tubes of lauryl tryptose broth, and return all the gas-negative tubes to the 35°C incubator for an additional 24 ± 2 hours.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Be sure to record all results under the 48-hour LTB column on the data sheet. Discard all tubes. The Presumptive Test is concluded at this point, and Presumptive coliforms per 100 ml can be computed according to the methods described elsewhere in this manual.

III TESTING TO CONFIRMED TEST STAGE

Note that the description starts with the sample inoculation and includes the Presumptive Test stage. The Confirmed Test preferred in Laboratories of this agency is accomplished by means of the brilliant green lactose bile broth (BGLB) and the acceptable alternate tests are mentioned in III F. In addition, the Fecal Coliform Test is included as an optional adjunct to the procedure.

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory, name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample. The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated.

NOTE: If 10-ml samples are being planted, it is necessary to use tubes containing the correct concentration of culture medium. This has previously been noted in II A-2.

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10-ml pipet for 10 ml sample portions, and 1-ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not remove the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1/2 inch into the sample; otherwise sample running down the outside of the pipet will make measurements inaccurate.

c When delivering the sample into the culture medium, deliver sample portions of 1 ml or less down into the culture tube near the surface of the medium. Do not deliver small sample volumes at the top of the tube and allow them to run down inside the tube; too much of the sample will fail to reach the culture medium.

d Prepare preliminary dilutions of samples for portions of 0.01 ml or less before delivery into the culture medium. See Table 1 for preparation of dilutions. NOTE: Always deliver diluted sample portions into the culture medium as soon as possible after preparation. The interval between preparation of dilution and introduction of sample into the medium never should be as much as 30 minutes.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5°C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Retain all gas-positive tubes of lauryl tryptose broth culture in their place in the rack, and proceed.

4 Select the gas-positive tubes of lauryl tryptose broth culture for Confirmed Test procedures. Confirmed Test procedures may not be required for all gas-positive cultures. If, after 24-hours of incubation, all five replicate cultures are gas-positive for two or more consecutive sample volumes, then select the set of five cultures representing the smallest volume of sample in which all tubes were gas-positive. Apply Confirmed Test procedures to all these cultures and to any other gas-positive cultures representing smaller volumes of sample, in which some tubes were gas-positive and some were gas-negative.

5 Label one tube of brilliant green lactose bile broth (BGLB) to correspond with each tube of lauryl tryptose broth selected for Confirmed Test procedures.

6 Gently shake the rack of Presumptive Test cultures. With a flame-sterilized inoculation loop transfer one loopful of culture from each gas-positive tube to the corresponding tube of BGLB. Place each newly inoculated culture into BGLB in the position of the original gas-positive tube.

7 After making the transfers, the rack should contain some 24-hour gas-negative tubes of lauryl tryptose broth and the newly inoculated BGLB.

8 If the Fecal Coliform Test is included in the testing procedures, consult Section V of this part of the outline of testing procedures.

9 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB an additional 24 ± 2 hours at 35° ± 0.5°C.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Some tubes will be lauryl tryptose broth and some will be brilliant green lactose bile broth (BGLB). Be sure to record results from LTB under the 48-hour LTB column and the BGLB results under the 24-hour column of the data sheet.

3 Label tubes of BGLB to correspond with all (if any) 48-hour gas-positive cultures in lauryl tryptose broth. Transfer one loopful of culture from each gas-positive LTB culture to the correspondingly-labeled tube of BGLB. NOTE: All tubes of LTB culture which were negative at 24 hours and became positive at 48 hours are to be transferred. The option described above for 24-hour cultures does not apply at 48 hours.

4 If the Fecal Coliform Test is included in the testing procedure, consult Section V of the part of the outline of testing procedures.

5 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB 24 ± 2 hours at 35° ± 0.5°C.

6 Discard all tubes of LTB and all 24-hour gas-positive BGLB cultures.

D 72-hour Procedures

1 If any cultures remain to be examined, all will be BGLB. Some may be 24 hours old and some may be 48 hours old. Remove such cultures from the incubator, examine each tube for gas production, and record results on the data sheet.

2 Be sure to record the results of 24-hour BGLB cultures in the "24" column under BGLB and the 48-hour results under the "48" column of the data sheet.

3 Return any 24-hour gas-negative cultures for incubation 24 ± 2 hours at 35 ± 0.5°C.

4 Discard all gas-positive BGLB cultures and all 48-hour gas-negative cultures from BGLB.

5 It is possible that all cultural work and results for the Confirmed Test have been finished at this point. If so, codify results and determine Confirmed Test coliforms per 100 ml as described in the outline on use of MPN Tables.

E 96-hour Procedures

At most only a few 48-hour cultures in BGLB may be present. Read and record gas production of such cultures in the "48" column under BGLB on the data sheet. Codify results and determine Confirmed Test coliforms per 100 ml.

F Streak-plate methods for the Confirmed Test, using eosin methylene blue agar or Endo agar plates, are accepted procedures in Standard Methods. The worker who prefers to use one of these media in preference to BGLB (also approved in Standard Methods) is advised to refer to the current edition of "Standard Methods for the Examination of Water and Wastewater" for procedures.

IV TESTING TO COMPLETED TEST STAGE

(Note that this description starts with the sample inoculation and proceeds through the Presumptive and the Confirmed Test stages. In addition, the Fecal Coliform Test is referred to as an optional adjunct to the procedure.)

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory, name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample. The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated. Guidance on labeling for laboratory data number and identification of individual tubes is described elsewhere in this outline.

NOTE: If 10-ml samples are being plated, it is necessary to use tubes containing the correct concentration of culture medium. This has previously been noted elsewhere in this outline and referral is made to tables.

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10-ml pipet for 10 ml sample portions, and 1-ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not move the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1/2 inch into the sample; otherwise sample running down the outside of the pipet will make measurements inaccurate.

c When delivering the sample into the culture medium, deliver sample portions of 1 ml or less down into the culture tube near the surface of the medium. Do not deliver small sample volumes at the top of the tube and allow them to run down inside the tube; too much of the sample will fail to reach the culture medium.

d Prepare preliminary dilutions of samples for portions of 0.01 ml or less before delivery into the culture medium. See Table 2 for preparation of dilutions. NOTE: Always deliver diluted sample portions into the culture medium as soon as possible after preparation. The interval between preparation of dilution and introduction of sample into the medium never should be as much as 30 minutes.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5°C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Retain all gas-positive tubes of lauryl tryptose broth culture in their place in the rack, and proceed.

4 Select the gas-positive tubes of lauryl tryptose broth culture for the Confirmed Test procedures. Confirmed Test procedures may not be required for all gas-positive cultures. If, after 24-hours of incubation, all five replicate cultures are gas-positive for two or more consecutive sample volumes, then select the set of five cultures representing the smallest volume of sample in which all tubes were gas-positive. Apply Confirmed Test procedures to all these cultures and to any other gas-positive cultures representing smaller volumes of sample, in which some tubes were gas-positive and some were gas-negative.

5 Label one tube of brilliant green lactose bile broth (BGLB) to correspond with each tube of lauryl tryptose broth selected for Confirmed Test procedures.

6 Gently shake the rack of Presumptive Test cultures. With a flame-sterilized inoculation loop transfer one loopful of culture from each gas-positive tube to the corresponding tube of BGLB. Place each newly inoculated culture into BGLB in the position of the original gas-positive tube.

7 If the Fecal Coliform Test is included in the testing procedure, consult Section V of this outline for details of the testing procedure.

8 After making the transfer, the rack should contain some 24-hour gas-negative tubes of lauryl tryptose borth and the newly inoculated BGLB. Incubate the rack of cultures at 35° C ± 0.5° C for 24 ± 2 hours.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Some tubes will be lauryl tryptose broth and some will be brilliant green lactose

bile broth (BGLB). Be sure to record results from LTB under the 48-hour LTB column and the BGLB results under the 24-hour column of the data sheet.

3 Label tubes of BGLB to correspond with all (if any) 48-hour gas-positive cultures in lauryl tryptose broth. Transfer one loopful of culture from each gas-positive LTB culture to the correspondingly-labeled tube of BGLB. NOTE: All tubes of LTB culture which were negative at 24 hours and became positive at 48 hours are to be transferred. The Option described above for 24-hour LTB cultures does not apply at 48 hours.

4 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB 24 ± 2 hours at 35° ± 0.5°C. Retain all 24-hour gas-positive cultures in BGLB for further test procedures.

5 Label a Petri dish preparation of eosin methylene blue agar (EMB agar) to correspond with each gas-positive culture in BGLB.

6 Prepare a streak plate for colony isolation from each gas-positive culture in BGLB on the correspondingly-labeled EMB agar plate.

Incubate the EMB agar plates 24 ± 2 hours at 35° ± 0.5°C.

D 72-hour Procedures

1 Remove the cultures from the incubator. Some may be on BGLB; several EMB agar plates also can be expected.

2 Examine and record gas production results for any cultures in BGLB.

3 Retain any gas-positive BGLB cultures and prepare streak plate inoculations for colony isolation in EMB agar. Incubate the EMB agar plates 24 ± 2 hours at 35 ± 0.5° C. Discard the gas-positive BGLB cultures after transfer.

4 Reincubate any gas-negative BGLB cultures 24 ± 2 hours at 35° ± 0.5°C.

5 Discard all 48-hour gas-negative BGLB cultures.

6 Examine the EMB agar plates for the type of colonies developed thereon. Well-isolated colonies having a dark center (when viewed from the lower side, held toward a light) are termed "nucleated or fisheye" colonies, and are regarded as "typical" coliform colonies. A surface sheen may or may not be present on "typical" colonies. Colonies which are pink or opaque but are not nucleated are regarded as "atypical colonies." Other colony types are considered "noncoliform." Read and record results as + for "typical" (nucleated) colonies ± for "atypical" (non-nucleated pink or opaque colonies), and - for other types of colonies which might develop.

7 With plates bearing "typical" colonies, select at least one well-isolated colony and transfer it to a correspondingly-labeled tube of lactose broth and to an agar slant. As a second choice, select at least two "atypical" colonies (if typical colonies are not present) and transfer them to labeled tubes of lactose broth and to agar slants. As a third choice, in the absence of typical or atypical coliform-like colonies, select at least two well-isolated colonies representative of those appearing on the EMB plate, and transfer them to lactose broth and to agar slants.

8 Incubate all cultures transfered from EMB agar plates 24 ± 2 hours at 35 ± 0.5°C.

E 96-hour Procedures

1 Subcultures from the samples being studied may include: 48-hour tubes of BGLB, EMB agar plates, lactose broth tubes, and agar slant cultures.

2 If any 48-hour tubes of BGLB are present, read and record gas production in the "48" column under BGLB. From any gas-positive BGLB cultures prepare streak plate inoculations for colony isolation on EMB agar. Discard all tubes of BGLB, and incubate EMB agar plates 24 ± 2 hours at 35 ± 0.5°C.

3 If any EMB plates are present, examine and record results in the "EMB" column of the data sheet. Make transfers to agar slants and to lactose broth from all EMB agar plate cultures. In decreasing order of preference, transfer at least one typical colony, or at least two atypical colonies, or at least two colonies representative of those on the plate.

4 Examine and record results from the lactose broth cultures.

5 Prepare a Gram-stained smear from each of the agar slant cultures, as follows:

NOTE: Always prepare Gram stain from an actively growing culture, preferably about 18 hours old, and never more than 24 hours old. Failure to observe this precaution often results in irregular staining reactions.

a Thoroughly clean a glass slide to free it of any trace of oily film.

b Place one drop of distilled water on the slide.

c Use the inoculation needle to suspend a tiny amount of growth from the nutrient agar slant culture in the drop of water.

d Mix the thin suspension of cells with the tip of the inoculation needle, and allow the water to evaporate.

e "Fix" the smear by gently warming the slide over a flame.

f Stain the smear by flooding it for 1 minute with crystal violet solution.

g Flush the excess crystal violet solution off in gently running water, and gently blot dry with filter paper or with other clean absorbent paper.

h Flood the smear with Lugol's iodine for 1 minute.

i Wash the slide in gently running water and blot dry with filter paper.

j Decolorize the smear with 95% alcohol solution with gentle agitation for 10-30 seconds, depending upon extent of removal of crystal violet dye, then blot dry.

k Counterstain for 10 seconds with safranin solution, then wash in running water and blot dry.

l Examine the slide under the microscope, using the oil immersion lens. Coliform bacteria are Gram-negative, nonspore-forming, rod-shaped cells, occurring singly, in pairs, or rarely in short chains.

m If typical coliform staining reaction and morphology are observed, record + in the appropriate space under the "Gram Stain" column of the data sheet. If typical morphology and staining reaction are not observed, then mark it ± or -, and make suitable comment in the "remarks" column at the right-hand side of the data sheet.

n If spore-forming bacteria are observed, it will be necessary to repurify the culture from which the observations were made. Consult the instructor, or refer to Standard Methods, for procedures.

At this point, it is possible that all cultural work for the Completed Test has been finished. If so, codify results and determine Completed Test coliforms per 100 ml.

F 120-hour Procedures and following:

1 Any procedures to be undertaken from this point are "straggler" cultures on media already described, and requiring step-by-step methodology already given in detail. Such cultures may be on: EMB plates, agar slants, or lactose broth. The same time-and-temperature of incubation required for earlier studies applies to the "stragglers" as do the observations, staining reactions, and interpretation of results. On conclusion of all cultural procedures, codify results and determine Completed Test coliforms per 100 ml.

V FECAL COLIFORM TEST

A General Information

1 The procedure described is an elevated temperature test for fecal coliform bacteria.

2 Equipment required for the tests are those required for the Presumptive Test of Standard Methods, a water-bath incubator, and the appropriate culture media.

B Fecal Coliform Test with EC Broth

1 Sample: The test is applied to gas-positive tubes from the Standard Methods Presumptive Test (lauryl tryptose broth), in parallel with Confirmed Test procedures.

2 24-hour Operations. Initial procedures are the planting procedures described for the Standard Methods Presumptive Coliform test.

a After reading and recording gas-production on lauryl tryptose broth, temporarily retain all gas-positive tubes.

b Label a tube of EC broth to correspond with each gas-positive tube of lauryl tryptose broth. The option regarding transfer of only a limited number of tubes to the Confirmed Test sometimes can be applied here. However, the worker is urged to avoid exercise of this option until he has assured the applicability of the option by preliminary tests on the sample source.

c Transfer one loopful of culture from each gas-positive culture in lauryl tryptose broth to the correspondingly labeled tube of EC broth.

d Incubate EC broth tubes 24 ± 2 hours at 44.5 ± 0.2°C in a waterbath with water depth sufficient to come up at least as high as the top of the culture medium in the tubes. Place in waterbath as soon as possible after inoculation and always within 30 minutes after inoculation.

3 48-hour operations

a Remove the rack of EC cultures from the waterbath, shake gently, and record gas production for each tube. Gas in any quantity is a positive test.

b As soon as results are recorded, discard all tubes. (This is a 24-hour test for EC broth inoculations and not a 48-hour test.)

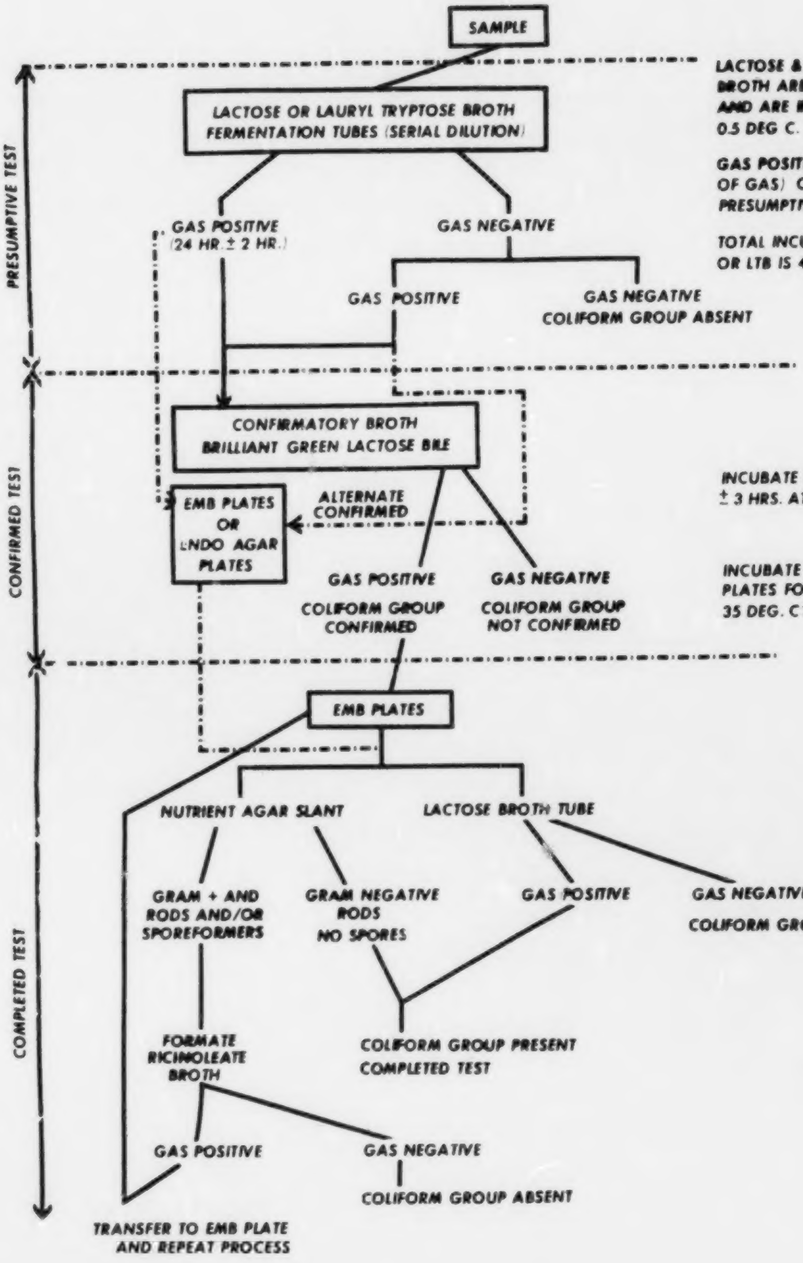
c Transfer any additional 48-hour gas-positive tubes of lauryl tryptose broth to correspondingly labeled tubes of EC broth. Incubate 24 ± 2 hours at 44.5 ± 0.2°C.

4 72-hour operations

a Read and record gas production for each tube. Discard all cultures.

b Codify results and determine fecal coliform count per 100 ml of sample.

TESTS FOR COLIFORM GROUP


SAMPLE
PRESUMPTIVE TEST
LACTOSE OR LAURYL TRYPTOSE BROTH FERMENTATION TUBES (SERIAL DILUTION)
GAS POSITIVE (24 HR. ± 2 HR.)
GAS NEGATIVE
GAS POSITIVE
GAS NEGATIVE COLIFORM GROUP ABSENT
CONFIRMED TEST
CONFIRMATORY BROTH BRILLIANT GREEN LACTOSE BILE
EMB PLATES OR ENDO AGAR PLATES
ALTERNATE CONFIRMED
GAS POSITIVE COLIFORM GROUP CONFIRMED
GAS NEGATIVE COLIFORM GROUP NOT CONFIRMED
COMPLETED TEST
EMB PLATES
NUTRIENT AGAR SLANT
LACTOSE BROTH TUBE
GRAM + AND RODS AND/OR SPOREFORMERS
GRAM NEGATIVE RODS NO SPORES
GAS POSITIVE
GAS NEGATIVE COLIFORM GROUP ABSENT
FORMATE RICINOLEATE BROTH
COLIFORM GROUP PRESENT COMPLETED TEST
GAS POSITIVE
GAS NEGATIVE
COLIFORM GROUP ABSENT
TRANSFER TO EMB PLATE AND REPEAT PROCESS

LACTOSE & LAURYL TRYPTOSE BROTH ARE INTERCHANGEABLE MEDIA AND ARE INCUBATED AT 35 DEG C ± 0.5 DEG C.

GAS POSITIVES TUBES (ANY AMOUNT OF GAS) CONSTITUTE A POSITIVE PRESUMPTIVE TEST

TOTAL INCUBATION TIME FOR LACTOSE OR LTB IS 48 HRS. ± 3 HRS.

INCUBATE BGLB TUBES FOR 48 HRS. ± 3 HRS. AT 35 DEG. C ± 0.5 DEG. C.

INCUBATE EMB OR ENDO AGAR PLATES FOR 24 HRS. ± 2 HRS AT 35 DEG. C ± 0.5 DEG. C.

Part 3
LABORATORY METHODS FOR FECAL STREPTOCOCCUS
(Day-By-Day Procedures)

I GENERAL INFORMATION

A The same sampling and holding procedures apply as for the coliform test.

B The number of fecal streptococci in water generally is lower than the number of coliform bacteria. It is good practice in multiple dilution tube tests to start the sample planting series with one sample increment larger than for the coliform test. For example: If a sample planting series of 1.0, 0.1, 0.01, and 0.001 ml is planned for the coliform test, it is suggested that a series of 10, 1.0, 0.1, and 0.01 ml be planted for the fecal streptococcus test.

C Equipment required for the test is the same as required for the Standard Methods Presumptive and Confirmed Tests, except for the differences in culture media.

II STANDARD METHODS (Tentative) PROCEDURES

A First-Day Operations

1 Prepare the sample data sheet and labeled tubes of azide dextrose broth in the same manner as for the Presumptive Test. NOTE: If 10-ml samples are included in the series, be sure to use a special concentration (ordinarily double-strength) of azide dextrose broth for these sample portions.

2 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

3 Measure the predetermined sample volumes into the labeled tubes of azide dextrose broth, using the sample measurement and delivery techniques used for the Presumptive Test.

4 Shake the rack of tubes of inoculated culture media, to insure good mixing of sample with medium.

5 Place the rack of inoculated tubes in the incubator at 35° ± 0.5° C for 24 ± 2 hours.

B 24-hour Operations

1 Remove the rack of tubes from the incubator. Read and record the results from each tube. Growth is a positive test with this test. Evidence of growth consists either of turbidity of the medium, a "button" of sediment at the bottom of the culture tube, or both.

2 Label a tube of ethyl violet azide broth to correspond with each positive culture of azide dextrose broth. It may be permissible to use the same confirmatory option as described for the coliform Confirmed Test, in this outline.

3 Shake the rack of cultures gently, to resuspend any living cells which have settled out to the bottom of the culture tubes.

4 Transfer three loopfuls of culture from each growth-positive tube of azide dextrose broth to the correspondingly labeled tube of ethyl violet azide broth.

5 As transfers are made, place the newly inoculated tubes of ethyl violet azide broth in the positions in the rack formerly occupied by the growth-positive tubes of azide dextrose broth. Discard the tubes of azide dextrose broth culture.

6 Return the rack, containing 24-hour growth-negative azide dextrose broth tubes and newly-inoculated tubes of ethyl violet azide broth, to the incubator. Incubate 24 ± 2 hours at 35° ± 0.5° C.

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C 48-hour Operations

1 Remove the rack of tubes from the incubator. Read and report results. Growth, either in azide dextrose broth or in ethyl violet azide broth, is a positive test. Be sure to report the results of the azide dextrose broth medium under the "48" column for that medium and the results of the ethyl violet azide broth cultures under the "24" column for that medium.

2 Any 48-hour growth-positive cultures of azide dextrose broth are to be transferred (three loopfulls) to ethyl violet azide broth. Discard all 48-hour growth-negative tubes of azide dextrose broth and all 24-hour growth-positive tubes of ethyl violet azide broth.

3 Incubate the 24-hour growth-negative and the newly-inoculated tubes of ethyl violet azide broth 24 ± 2 hours at 35° ± 0.5°C.

D 72-hour Operations

1 Read and report growth results of all tubes of ethyl violet azide broth.

2 Discard all growth-positive cultures and all 48-hour growth-negative cultures.

3 Reincubate any 24-hour growth-negative cultures in ethyl violet azide broth 24 ± 2 hours at 35° ± 0.5°C.

E 96-hour Operations

1 Read and report growth results of any remaining tubes of ethyl violet azide broth.

2 Codify results and determine fecal streptococci per 100 ml.

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DETERMINATION OF BACTERIAL POLLUTION

Multiple Dilution Tube Methods

I INTRODUCTION

The basic procedures of multiple dilution tube methods, often called the Most Probable Number (MPN) Method, are described here, as applied in tests for the coliform group, fecal coliform group, and for the fecal streptococci. The methods are described in greater detail elsewhere in this course manual.

II PRINCIPLES OF MULTIPLE DILUTION TUBE METHODS

A Basic Assumptions of the Method

1 Bacteria in a sample can be freed from one another by shaking, so that single-cell units are uniformly dispersed in the sample.

2 Through inoculation of progressively smaller volumes of sample into a series of tubes of culture medium, some tubes will receive single-cell units of the test bacteria and some will not.

3 Culture media being used in the procedure will permit growth of all cells of the organisms for which the test is designed.

4 The testing procedure will give a simple "yes" (positive test) or "no" (negative test) answer for each inoculated tube, with respect to presence of the organism for which the test has been devised.

5 Probability mathematics can be applied to the results of the testing procedure to give quantitative information (Most Probable Number) on the density of the test organisms in the sample source.

B Application

1 Quantitative application

Most probable number methods can be applied in almost any conceivable combination of sample inoculation volumes and number of replicate tubes per volume. Standard practice of this organization in water pollution surveys is to inoculate 5 replicate tubes in a series of sample volumes in successively decreasing decimal increments. At one time it was common to use 3 tubes per decimal sample increment, but due to the relative lack of precision of results, the practice has been largely abandoned.

2 Qualitative aspects

a To assure growth of the test organisms, the tests are performed in stages. The stages of the Standard Coliform Test are the well-known Presumptive, Confirmed, and Completed Tests, respectively.

b The fecal coliform test also is performed in stages, using the standard Presumptive Test as the first stage, and a special confirmatory procedure to differentiate between total coliforms and fecal coliforms.

c Similarly, fecal streptococcus tests are conducted in two stages, with the first stage serving primarily as an enrichment and limited differential test which is further verified by results from the second, more selective, culture medium.

III STANDARD COLIFORM TEST

A Presumptive Test

1 Application

The Presumptive Test is the primary stage of the procedures leading to the Confirmed Test and the Completed Test.

The test alone is not applicable in testing potability of drinking water, but it may be used for intermediate process control in treatment plants, or on occasion, in the study of waters known to be heavily polluted.

2 Culture medium

The bacteriologist has a choice between standard lactose broth and lauryl tryptose lactose broth, in fermentation tubes.

Lauryl tryptose lactose broth is preferred. This medium is more selective for coliform bacteria, and it exerts no apparent suppressive effect on any strains of the coliform group.

3 Incubation

In this and in all the Standard Methods test procedures for coliforms, incubation is at $35^{o}C \pm 0.5^{o}C$. This temperature is fixed arbitrarily. Cultures may be incubated up to 48 ± 3 hours. Continued incubation of positive tubes is unfavorable to coliform bacteria. Cultures are examined for the development of positive tests at the 24-hour interval and negative cultures are returned to the incubator for the remainder of the incubation period. Tubes giving positive results are subject to the Confirmed Test.

4 Procedure

Predetermined volumes of sample are measured into the culture medium and incubated up to 48 hours. Fermentation tubes showing any amount of visible gas are reported as positive tests. Gas formation is another criterion for coliforms.

B Confirmed Test

1 Application

The Confirmed Test is the procedure applied to tubes giving positive results in the Presumptive Test. To lighten the work-load, it may be permissible to subject to the Confirmed Test only certain positive Presumptive Test tubes. Our usual practice is to apply the Confirmed Test to all tubes representing the smallest sample volume in which all replicate sample increments gave positive results, and to all positive tubes representing lesser sample volumes. A preponderance of coliform organisms is assumed.

All tubes giving positive results at 48 hours but not at 24 hours are subjected to the Confirmed Test. Slow fermenters are more likely to be noncoliforms.

The test may be used for routine potability tests on drinking waters, on bathing waters, and on chlorinated effluents from sewage treatment plants.

When the MPN procedure is used for coliform tests in stream surveys, the Confirmed Test is the most-used routine method.

It is strongly urged, however, that the worker continue a limited number of tests to the Completed Test stage in order to demonstrate the applicability of the Confirmed Test. This is especially important with waters known to receive appreciable amounts of land drainage. If there is unacceptable discrepancy between the results obtained by the two methods, high quality work will require use of the more cumbersome Completed Test for the entire survey.

2 Culture media

The bacteriologist has a choice among three different media for the Confirmed Test. These are, brilliant green lactose bile broth, eosin methylene blue agar plates, and Endo agar.

Brilliant green lactose bile broth in fermentation tubes is the Confirmed Test medium of choice in most laboratories.

3 Incubation

In order to show distinctive colony form, the agar plate media are incubated up to 24 hours.

4 Procedure notes

If brilliant green lactose bile broth is used, one loopful of the culture from each positive Presumptive Test tube selected for confirmation is adequate inoculum. Within 48 hours, coliforms should form gas in some amount.

Streaking is done with an inoculating needle, so manipulated as to ensure development of several separated colonies. After incubation the colony morphology of separated colonies is studied. Colonies having nucleated centers, with or without surface sheen, are reported as positive tests. Opaque or mucoid colonies lacking nuclei are considered atypical coliforms. Other types of colonies are reported as negative tests.

C Completed Test

1 Application

The Completed Test is the official coliform test of Standard Methods, and is the test to be used on all water potability tests, subject to the exceptions noted in the Confirmed Test.

The test is applied to all positive tests from the Confirmed Test and to all atypical results noted when eosin methylene blue agar or Endo agar plates are used. The test determines whether bacteria in question are or are not coliforms by definition.

2 Culture media

If brilliant green lactose bile broth is used for the Confirmed Test, then eosin methylene blue agar plates or Endo agar plates are needed. In addition, nutrient agar slants and lactose broth fermentation tubes are needed.

3 Procedure notes

If brilliant green lactose bile broth was used in the Confirmed Test, then pure cultures must be obtained by the streaking procedures.

Typical coliform colonies, or atypical colonies if typical colonies are not present, are fished from one or more plates. Tubes of lactose broth and nutrient agar slants are then inoculated for gas production and Gram stain reaction.

Smears for Gram stains should be prepared from all nutrient agar slant cultures within an incubation period of 24-hours because Gram reaction may become variable. Incubation of the lactose broth cultures is up to 48 hours. If gas is produced in any quantity, this portion of the Completed Test is positive, and Gram stains are made of the corresponding smears.

A positive test requires compliance with the coliform definition in all details.

IV FECAL COLIFORM TEST

A Application

1 The significance of this test is discussed in the outline on bacterial indicators of pollution. It should be recalled that this test once was regarded by many as a specific test for E. coli.

2 The test is applied to the same positive Presumptive Test tubes of the standard coliform test that are subjected to the standard Confirmed Test procedures. The Presumptive tube enrichment elevates test sensitivity. With some samples experience will demonstrate need for applying the test to all positive Presumptive Test cultures.

B Procedure

1 One standard loopful from each positive Presumptive Test culture to be tested is transferred to EC Broth in fermentation tubes, and incubated for 24 ± 2 hours at 44.5°C ± 0.2°C in a forced circulation water bath.

2 Gas in any quantity is a positive test. Experimentally fecal coliforms give gas; other bacteria do not.

V FECAL STREPTOCOCCUS TEST

A The status of this group as pollution indicators is discussed in the consideration of bacterial indicators of pollution.

B Method of Litsky, Mallman, and Fifield

1 Presumptive Test

Predetermined sample increments are delivered into azide-dextrose broth. Turbidity in the medium indicates growth and constitutes a positive test. Azide is a strong inhibitor and few bacteria grow besides streptococci. Azide also slows streptococcus growth so that 48 hours incubation is necessary.

2 Confirmatory Test

Three loopfuls of culture from positive presumptive test tubes are transferred to tubes of ethyl violet azide broth, and incubated up to 48 hours at 35°C. Three loopfuls are to overcome medium inhibition.

Development of turbidity in the medium indicates growth and is a positive test.

The original research in development of this method required microscopic examination of Gram-stained smears of cultures. Standard Methods, 13th edition, does not require staining.

Membrane Filter Procedures

I INTRODUCTION

A Scope

1 The membrane filter, as currently used in our laboratories, consists of a porous disk of cellulose esters 47 mm in diameter and approximately 0.15 mm thick. Filters used in water bacteriology have pores of 0.45 microns in diameter. The basic principles of use in water bacteriology are as follows:

a A sterile membrane filter is fastened into a suitable filter holding device and a predetermined volume of a water sample is filtered through the membrane filter.

b The filter is placed in a culture container either on a paper pad impregnated with moist culture medium or upon an agar medium.

c The inoculated filter is incubated under prescribed conditions of temperature and humidity for a designated time.

d After incubation, the resulting culture is examined, and the necessary interpretations and/or additional tests are made.

2 Through variations in such factors as the composition of the culture medium, incubation time and temperature, and combinations with other cultural and biochemical tests, several kinds of bacterial determinations are possible.

B Basic Principles and Origins of Membrane Filter Techniques

The purpose of this discussion is to provide orientation in the types and amount of equipment and supplies needed in membrane filter analyses in water quality surveys, the basic methodology, limitations of membrane filter procedures, and the status of membrane filter methods in water quality testing. Finally, it considers the level of technical skill and the workloads of personnel assigned to perform tests by this method.

II REQUIREMENTS FOR EQUIPMENT AND SUPPLIES

A Laboratory Supplies and Equipment

1 Membrane filters

a Membrane filters are available from at least 3 different commercial sources, representing the United States, Britain, and Germany. Each source applies a distinctive tradename to its MF product:

"Millipore Filters" - Millipore Filter Corp., U. S. A.

"Bac-T-Flex Filters" - Schleicher and Schuell, U. S. A. representatives for Sartorius - Werke, Germany.

"Oxoid Filters" - Oxo Limited, Britain.

b Membrane filters are available in a wide variety of controlled pore sizes. Each commercial source will designate the filter most suitable for water bacteriological tests upon request.

2 Absorbent pads for nutrients

Absorbent paper disks for mechanical support of the filters during incubation and for maintaining an adequate supply of culture medium in contact with the filters, ordinarily are sold with the membrane filters. Additional supplies are obtainable from filter manufacturers.

3 Filter-holding units

a The filter-holding unit is a device for supporting the membrane filter and for holding the sample until it passes through the filter. During the filtration, the sample passes through a circular area, usually about 35 mm in diameter, in the center of the filter. The outer part of the filter disk is clamped between the two essential components of the filter-holding unit.

1) The lower element, called the filter base, or receptacle, supports the membrane filter on a plate about 50 mm in diameter. The central part of this plate is porous, to allow free passage of liquid. The outer part of the plate is a smooth nonporous surface. The lower element includes fittings for mounting the unit in a suction flask or other container suitable for filtration with vacuum.

2) The upper element, usually called the funnel, holds the sample until it is drawn through the filter. Its lower portion is a flat ring that rests directly over the nonporous part of the filter support plate.

3) When assembled, the two elements of the filter-holding unit are joined by a locking ring or by one or more clamps or springs.

b Filter-holding units are either metal or glass. Metal units are available from at least six commercial sources, and glass units are available from at least two sources. Most workers prefer to use metal filter-holding units, due to their greater durability. Most of the use of glass units seems to be dictated by economic considerations, as these are much lower in initial cost.

4 Culture media

a Culture media are commercially available for use with membrane filters in the determination of bacterial plate counts, for coliform counts, fecal coliform counts, fecal streptococcus counts and, in addition, for several special applications.

b These media can be obtained in several forms, including:

1) Dehydrated media, requiring weighing, addition of a suitable amount of distilled water, and some form of sterilization prior to use. This form of media is preferred as it appears to give the most satisfactory combination of performance quality, stability and economy.

2) Ready-to-use culture media, sealed in glass ampoules, and

3) Dehydrated culture media, impregnated in absorbent pads. Media in this form require only the addition of a suitable amount of sterile distilled water to the paper pad to be ready for use. These media have limited shelf life, and are not widely used at this time.

5 Culture containers

Several forms of culture containers are suitable for membrane filter work. These include small glass Petri dishes, and plastic Petri dishes. The plastic Petri dishes may be preferred, as they are sufficiently cheap to encourage single-service use.

6 Vacuum sources

In the fixed laboratory, central vacuum service, laboratory-scale electric vacuum pumps, or water pumps ("aspirators") are suitable for vacuum filtration of samples.

7 Glassware

Conventional laboratory glassware is used in fixed laboratories for measurement and delivery of samples for filtration, sample dilutions, and the like. Under field conditions it sometimes is necessary to use a limited amount of laboratory glassware, with some form of field application of sterilization methods.

8 General laboratory facilities

Such facilities as autoclave, incubators, weighing equipment, and the like are required in conventional bacteriology laboratories, and are needed equally when membrane filter methods are used.

B Field Equipment

Field operations cannot be carried on indefinitely without support from a central laboratory. Functions such as preparation of distilled water, pre-weighing of culture media, sterilization of membrane filters and culture containers, and other general preparations are best performed in a central laboratory facility.

1 With the introduction of membrane filters to the bacteriological analysis of water, a number of investigators have given attention to the development of membrane filter equipment which can be used in the field in easily portable, self-contained water laboratories. Such portable laboratory kits would have three major fields of usefulness:

a They would be useful in certain routine water quality control operations. Examples include such places as on board ships, in some national parks, on airlines, etc.

b They could be used in certain types of stream surveys, where there is an excessive delay between sample collection and the time when examination can be started, due to time required for transportation of samples.

c Finally, such units would be invaluable in emergencies, when existing laboratories are overburdened or inoperative. Portable kits already have proven extremely useful in testing many small water supplies in a short period of time. Further, there is a predictable need for such equipment in the event of natural or wartime civil defense disaster.

2 Availability of field equipment

Commercially development equipment is available from several sources.

a Equipment of this type includes provision for sample filtration, field sterilization (where necessary) of filtration equipment, limited capacity for reserves of culture media and other supplies, and provision for incubation of cultures. Such equipment does not include satisfactory visual aid equipment (a low powered microscope is needed for streptococcus counts, and at least a simple lens is needed for coliform work), and provision for many of the supplies is not included.

b Performance of the various forms of commercially available field equipment is not equal. Buyers of field equipment are urged to study the equipment specifications quite carefully, and if necessary, perform comparative tests with laboratory type equipment to determine the type of field equipment most suited to their own needs.

III MEMBRANE FILTER PROCEDURES IN WATER QUALITY SURVEYS

A Standard Coliform Test

1 Basic procedures

a Sample filtration volumes are selected (usually 3 volumes) to give at least one volume which will contain 20-80 typical coliform colonies.

b The sample volumes are filtered through membrane filters, and incubated at 35°C for 20-22 hours in an atmosphere at or near saturation.

c Following incubation, the typical coliform colonies are counted and reported as coliforms per 100 ml of sample.

2 Applications

The total coliform count has been used most widely of all procedures when bacteriological data is collected in a survey. Applications of membrane filter methods in surveys necessarily have a short history. The method has been used in certain studies of the Potomac River System, in the Illinois-Great Lakes studies, and in certain surveys in the West.

The Standard MF coliform test is applicable when it can be initiated within the recommended period between sample collection and receipt at the laboratory. The procedure is applicable with MF field units.

B "Fecal Coliform" Test with Membrane Filters

1 Procedure

a Sample filtration procedures are the same as for the Standard Membrane Filter coliform test. Sample filtration volumes should be selected so that at least one filter will produce 20-60 fecal coliform colonies.

b The culture medium is M-FC Broth. The medium requires addition of a rosolic acid solution prior to use.

c Within 20 minutes after sample filtration, the membrane filters are placed in small plastic bags, which are sealed, and immersed in a forced circulation water bath at 44.5°C ± 0.2°C for a 24 hour incubation time.

d Fecal foliform colonies are blue or blue-hues, generally 1 - 3 mm in diameter. The fecal coliform density is reported as fecal coliforms per 100 ml of sample.

2 Application

The fecal foliform test is being used increasingly in water quality studies for improved evidence of recent direct origin of intestinal pollution in the water samples being tested.

C Fecal Streptococcus Tests

1 Basic procedures

a The filtration procedures are the same as with the coliform tests. The sample volumes should be such that at least one membrane filter will produce 20 - 100 fecal streptococcus colonies.

b The culture medium can be the M-Enterococcus Agar developed by Slanetz and his associates, or it can be KF-Broth, developed by Kenner and his associates. In either case, incubation is 48 hours at 35°C.

c With both media, fecal streptococcus colonies are pale pink to deep red, up to 2 mm in diameter.

d Because the density of fecal streptococci in most waters is lower than coliform density, it is necessary to filter greater volumes of water. Further, the number of colonies that can be counted effectively is greater than the number of coliform colonies. Sample volumes often can be 10 to 100 -fold greater for fecal streptococcus counts than for coliform counts.

2 Application

Fecal streptococcus counts are especially useful when supplementary data will support information from the coliform counts. Fecal streptococcus counts are used in many states in addition to coliform tests in examination of bathing waters as well as in stream surveys.

IV LIMITATIONS OF MEMBRANE FILTER METHODS

Most of the recognized limitations of the membrane filter method have been related to tests for members of the coliform group. Extension of these recognized limitations to tests for other pollution indicators requires special interpretation.

A Need for Selection of Suitable Filtration Volume

1 In tests for coliform bacteria, the sample filtration volumes should be so selected that one of volumes yields 20-80 coliform colonies. With fewer than 20 colonies, the random variation of colony counts becomes great enough to cause some difficulty in statistical analysis; with more than 80 coliform colonies, the proportionality between number of coliform colonies differentiated and sample volume begins to fail. This is especially troublesome in waters containing large numbers of noncoliform bacteria capable of growing on the filters.

2 In tests for the fecal streptococci, fewer nonstreptococcus interference colonies are capable of growing on the medium. For this reason and because fecal streptococcus colonies are small, the sample filtration volumes are so selected as to yield 20-100 colonies on a membrane filter.

B Influence of Turbidity

When the water being studied contains large amounts of suspended matter, such as clay, and has relatively low counts of the indicator organisms being studied, cultural difficulties often result. The particulate matter suspended in the water is deposited on the surface of the membrane filter in sample filtration, with any bacteria contained in the sample. Culture medium diffusing through the membrane filter forms a capillary layer around all the particles of matter deposited on the filter. This results in development of spreading, poorly defined bacterial colonies.

The solutions to problems of this type are: 1) to filter the sample in several smaller increments so that the combined sample filtration volume is spread over several membrane filters, each of which has a lesser amount of particulate matter deposited on the surface; or, 2) perform the analysis by means of the dilution tube (MPN) procedure.

C Influence of large numbers of bacteria representing forms other than the indicator group being studied.

1 In coliform testing, this can be a serious problem. The difficulty arises when appreciable numbers of noncoliform bacteria are present and are capable of growing on the Endo-type culture medium used. When large numbers of noncoliform bacteria grow on the medium, and few coliform

bacteria are present, the sheen-producing capacity of many of the coliform colonies is impaired, and the result indicates the presence of fewer coliform bacteria than actually are present.

A solution for this type of problem is to filter several smaller sample increments, or else to use the MPN method.

2 In tests for fecal streptococci, the presence of large numbers of bacteria of other groups has not proven troublesome. The medium is extremely selective, and the few forms that do seem to grow on the medium produce extremely small colonies which have not been shown to have any adverse effect on the qualitative results obtained in tests for fecal streptococci.

V OFFICIAL STATUS OF MEMBRANE FILTER METHODS

A American Public Health Association, American Water Works Association, and Water Pollution Control Federation, in "Standard Methods for the Examination of Water and Wastewater." (13th Edition, 1971)

1 A single-stage procedure, using M-Endo Broth MF for tests for members of the coliform group, is described as an official testing procedure.

2 A new two-stage procedure has been added.

3 A delayed incubation procedure, using benzoated M-Endo Broth MF, is designated a Tentative Method.

4 A test for enterococci (fecal streptococci), using M Enterococcus Agar or KF broth, is designated a Tentative Method.

B The Technical Committee on Methods for the Bacteriological Examination of Boundary Waters recommended use of membrane filter methods for the examination of boundary waters. The Advisory Committee adopted the recommendation in January, 1957.

C The U.S. Government, in Interstate Quarantine Drinking Water Standards

1 (Federal Register, October 23, 1956, pp. 8110-11 and March 1, 1957, Vol. 22, p. 1271). Preliminary and final authorization was granted to use membrane filter coliform tests for the examination of potable waters at interstate carrier water points and other waters under water quality control regulations of the Public Health Service or other agencies of the U.S. Government.

Supplemental Tests

I INTRODUCTION

A Need for Supplemental Tests

1 To demonstrate applicability of nonstandard procedures to the particular survey situation.

2 To clarify significance of results obtained through application of standard procedures.

3 To investigate anomalous results obtained by routine survey procedures, such as evidence of toxic substances in the water, unexplained high densities of pollution indicators, etc.

4 To develop information not otherwise shown by the routine testing program.

B Scope

This discussion is concerned with further differentiation of members of the coliform group, fecal streptococcus group, enteric pathogens, and with a bioassay test in which bacteria are used as the test organism.

II IMViC TESTS

A Nature of Tests

1 Pure cultures for IMViC classification are obtained from agar plate or from membrane filter isolations from the sample source. For development of relative numbers of the various IMViC types, it never is acceptable to use culture isolates from the dilution tube method.

2 "IMViC" is a mnemonic expression, referring to a series of four biochemical tests. The tests and basic principles of each are as follows:

a Indole test (I):

This is a test to determine if indole is produced as an end-product of the utilization of the amino acid tryptophane. The culture is transferred to tryptophane broth and incubated 24 hours, after which 4 ml of the culture is tested with Kovac's reagent. Development of a cherry-red color in the reagent indicates presence of indole.

b Methyl red test (M):

This is a test of the terminal pH of the culture after utilization of a fermentable carbohydrate, glucose. The culture is incubated 5 days in a standard peptone-glucose medium, after which a few drops of methyl red indicator are added. Development of a red color constitutes a positive test, demonstrating that the organism being tested has brought the medium to a more strongly acid condition than is obtained with a negative result (development of a yellow color).

c Voges-Proskauer test (V):

This is to determine whether acetylmethylcarbinol is produced as one of the end-products of the medium used for the methyl red test. After 48 hours of incubation, 5 ml of the medium is removed from the culture and tested with a-naphthol solution. The presence of acetylmethylcarbinol is indicated by development of a red color.

d Citrate test (C):

This test is to determine whether the strain of coliform organisms being tested is capable of using citrate as the sole source of carbon. A small inoculum of culture is transferred to Koser's citrate medium or

Simmons' citrate agar. Growth in or on either medium is evidence that the organism can use citrate and constitutes a positive test.

B Application of IMViC Tests

1 Field survey workers make little routine use of IMViC testing procedures in water pollution surveys. Essentially the same information relative to intestinal origin of coliforms is obtainable on a quantitative basis by the fecal coliform test.

2 In the event that appreciable amounts of land drainage are to be considered in a survey, IMViC classification may be useful, as a means of recognizing IMViC types of coliforms which may become predominant in soils (-+-+).

3 IMViC classification is a useful research laboratory tool as a means of segregating pure cultures of coliform bacteria into groups having a similar biochemical and behavioral characteristics. It is noted that in theory 16 IMViC categories are identifiable.

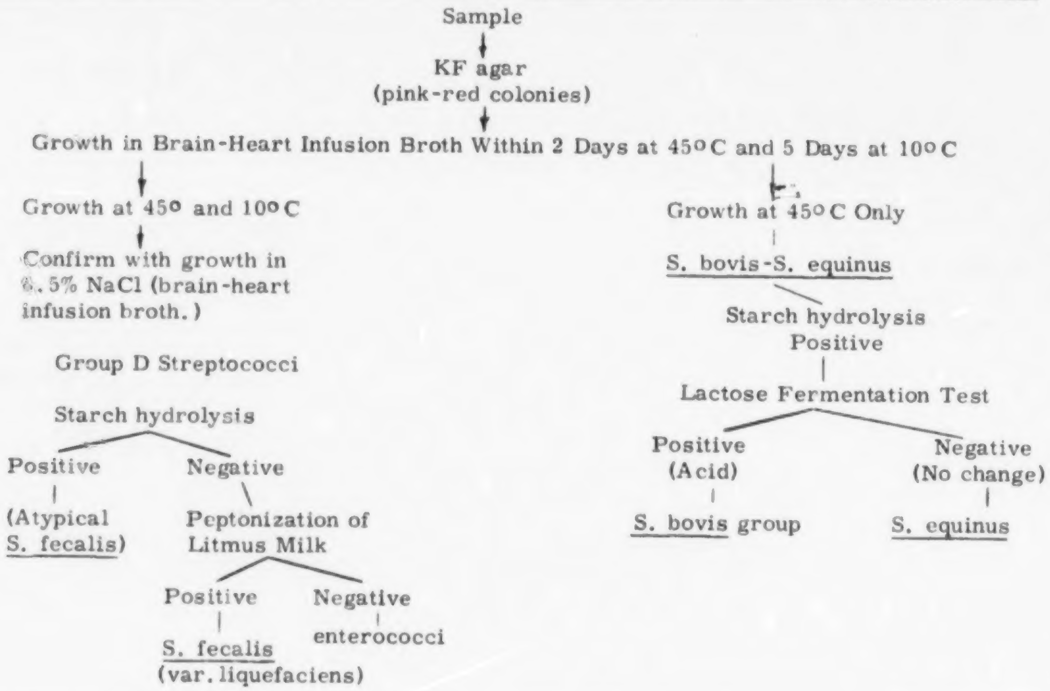
III PARTITION COUNTING OF STREPTOCOCCUS SPECIES

A Nature of Tests

1 Tests are applied to pure cultures obtained from agar plates or membrane filter isolations from the sample source. As with IMViC tests, partition counts are not acceptable if made from dilution tube isolates.

2 A series of biochemical tests is made which lead to species or species-group identification of each culture. A simplified protocol for these tests is shown in the following table:

SCHEMATIC OUTLINE FOR IDENTIFICATION OF FECAL STREPTOCOCCI


Sample
KF agar
(pink-red colonies)
Growth in Brain-Heart Infusion Broth Within 2 Days at 45°C and 5 Days at 10°C
Growth at 45° and 10°C
Confirm with growth in 6.5% NaCl (brain-heart infusion broth.)
Group D Streptococci
Starch hydrolysis
Positive
(Atypical S. fecalis)
Negative
Peptonization of Litmus Milk
Positive
S. fecalis (var. liquefaciens)
Negative
enterococci
Growth at 45°C Only
S. bovis-S. equinus
Starch hydrolysis Positive
Lactose Fermentation Test
Positive (Acid)
S. bovis group
Negative (No change)
S. equinus

NOTE: A small percentage of unclassified strains will be found since fecal streptococci vary greatly in their biochemical reactions.

B Applications

As described in the discussion of indicator organisms, certain streptococcal species are normal inhabitants of the intestines of domestic animals; others are not. The tests are made to account for probable human vs. probable animal origin of pollution-indicating bacteria found in water samples.

IV DEMONSTRATION OF ENTERIC PATHOGENS

A Nature of Tests

1 The tests are performed in multiple stages, and involve a combination of cultural biochemical tests and serological identification procedures. The essential steps are:

  a Application of special sample collection procedures such as filtration or gauze pad concentration

  b Enrichment of sample and primary isolation of cultures.

  c Tentative identification by cultural determination of biochemical properties.

  d Confirmation and completion of identification by serological procedures.

2 The details of these tests are far beyond the scope of this discussion. The interested worker should consult a standard reference on the subject. "Identification of Enterobacteriaceae" by P. R. Edwards and W. H. Ewing (Burgess Publishing Co., Minneapolis 15, Minnesota) is particularly recommended.

B Application of Tests

In certain water pollution studies, survey workers currently are making tests to show presence of species of Salmonella as positive evidence of microbiological hazard in the waters being tested. In this connection the following points are emphasized:

1 Demonstration of presence of these organisms is a qualitative, not a quantitative, procedure.

2 Salmonella species, when demonstrated, may or may not be of human origin; however, any Salmonella species demonstrated is regarded as a potential hazard to human health since all are pathogenic.

3 Tests to determine the presence of enteric pathogens are supplemental, not alternatives, to tests for the established bacterial indicators of pollution.

4 The tests require a high order of skill and training, as well as special supplies, beyond the present resources of most water laboratories.

V TOXICITY TESTING OF WATER

A Nature of Test

1 Contrary to all the other tests described, this test does not necessarily involve laboratory work on organisms recovered from the water source, but is a test of the water itself. The water is used as solvent for a synthetic culture medium, and used to cultivate a pure culture of a strain of Aerobacter aerogenes. A parallel synthetic culture medium is tested in a similar manner, but distilled water of the highest purity is used in media preparation. After 24 hours of incubation, bacterial densities from both media are compared.

2 Through various combinations of the constituents of the culture medium, the worker can determine whether substances unfavorable to the test organism are present, or he can demonstrate the possible presence of nutritive materials in the water source, which might help to account for unexpected elevations of density of the pollution indicator.

3 The details of the test correspond quite closely to the test for distilled water quality, described in the current (13th) edition of "Standard Methods for the Examination of Water and Wastewater."

B Applications: The Test Has Potential Use In

1 Studies in which pollution-indicating bacteria seem to disappear more rapidly than expected, on the basis of experience with environmental waters in general, through demonstration of bactericidal or bacteriostatic properties of the test water; or

2 In studies in which elevations of the density of pollution indicators occur and there is suggestion of prolonged aftergrowth of the organisms due to high levels of available nutrient. This type of test must be undertaken with great caution, as the testing procedure removes many of the environmental factors which tend to cause disappearance of the pollution indicators, notably predatory organisms, other microorganisms which compete more favorably for the available food supply, and certain chemical substances.

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Descriptors: Coliforms, Fecal Coliforms, Fecal Streptococci, Filters, Indicator Bacteria, Laboratory Equipment, Laboratory Tests, Membranes, Microbiology, MPN, Most Probable Number, Sewage Bacteria, Water Analysis

MEMBRANE FILTER LABORATORY AND FIELD PROCEDURES

I BASIC PROCEDURES

A Introduction

Successful application of membrane filter methods requires development of good routine operational practices. The detailed basic procedures described in this Section are applicable to all membrane filter methods in water bacteriology for filtration, incubation, colony counting, and reporting of results. In addition, equipment and supplies used in all membrane filter procedures are described here and not repeated elsewhere in such detail.

Workers using membrane filter methods for the first time are urged to become thoroughly familiar with these basic procedures and precautions.

B General Supplies and Equipment List

Table 1 is a check list of materials.

C "Sterilizing" Media

Set tubes in a boiling waterbath for 10 minutes. This method suffices for medium in tubes up to 25 × 150 mm. Frequent agitation improves dissolving of the medium.

Alternately, coliform media can be directly heated on a hotplate to the first bubble of boiling. Stir the medium frequently if direct heat is used, to avoid charring the medium.

Do not autoclave.

D General Laboratory Procedures with Membrane Filters

1 Prepare data sheet

Minimum data required are: sample identification, test performed including media and methods, sample filtration volumes, and the bench numbers assigned to individual membrane filters.

2 Disinfect the laboratory bench surface.

Use a suitable disinfectant solution and allow the surface to dry before proceeding.

3 Set out sterile culture containers in an orderly arrangement.

4 Label the culture containers.

Numbers correspond with the filter numbers shown on the data sheet.

5 Place one sterile absorbent pad* in each culture container, unless an agar medium is being used.

Use sterile forceps for all manipulations of absorbent pads and membrane filters. Forceps sterility is maintained by storing the working tips in about 1 inch of methanol or ethanol. Because the alcohol deteriorates the filter, dissipate it by burning before using the forceps. Avoid heating the forceps in the burner as hot metal chars the filter.

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*When an agar medium is used, absorbent pads are not used. The amount of medium should be sufficient to make a layer approximately 1/8" deep in the culture container. In the 50 mm plastic culture containers this corresponds to approximately 6-8 ml of culture medium.

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Office Of Water Programs, Environmental Protection Agency.

Table 1. EQUIPMENT, SUPPLIES AND MEDIA (Cont'd)

Item	Standard Tests		Nonstandard Tests			
	M-Endo Broth	L. E. S. Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Test
Half-round glass paper weights for colony counting, with lower half of a 2-oz metal ointment box	X	X	X	X	X	
Hand tally, single unit acceptable, hand or desk type	X	X	X	X	X	
Stereoscopic (dissection) microscope, magnification of 10X or 15X, preferable binocular wide field type	X	X	X	X	X	X
Bacteriological inoculating needle						X
Wire racks for culture tubes, 10 openings by five openings preferred, dimensions overall approximately 6" × 12"						X
Phenol Red Lactose Broth in 16 X 150 mm fermentation tubes with metal caps, 10 ml per tube						X
Eosin Methylene Blue Agar (Levine) in petri plates, prepared ready for use						X
Nutrient agar slants, in screw capped tubes, 15 × 126 mm						X
Gram stain solutions, 4 solutions per complete set						X
Microscope, compound, binocular, with oil immersion lens, microscope lamp and immersion oil						X
Microscope slides, new, clean, 1" × 3" size						X
Water proof plastic bags for fecal coliform culture dish incubation				X		
M-Endo medium, MF dehydrated medium in 25 × 95 mm flat bottomed screw-capped glass vials, 1.44 g per tube, sufficient for 30 ml of medium	X		X			
Ethanol, 95% in small bottles or screw-capped tubes, about 20 ml per tube	X	X	X			
Sodium benzoate solution, 12% aqueous, in 25 × 150 mm screw-capped tubes, about 10 ml per tube			X			
L. E. S. Endo Agar MF, dehydrated M-Endo medium, 0.36 g per 25 × 95 mm flat bottomed screw-capped glass vial, plus 0.45 g agar, for 30 ml		X				
Lactose Lauryl Sulfate Tryptose Broth in 25 × 150 mm test tube without included gas tube, about 25 ml, for enrichment in L. E. S. method		X				

Table 1. EQUIPMENT, SUPPLIES AND MEDIA

	Standard Tests		Nonstandard Tests			
Item	M-Endo Broth	L. E. S. Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Test
Funnel unit assemblies	X	X	X	X	X	
Ring stand, with about a 3" split ring, to support the filtration funnel	X	X	X	X	X	
Forceps, curved-end round tipped, special type for MF work	X	X	X	X	X	
Methanol, in small wide-mouthed bottles, about 20 ml for sterilizing forceps	X	X	X	X	X	
Suction flasks, glass, 1 liter, mouth to fit No. 8 stopper	X	X	X	X	X	
Rubber tubing, 2-3 feet, to connect suction flask to vacuum services, latex rubber 3/16" I. D. by 3/32" wall	X	X	X	X	X	
Pinch clamps strong enough for tight compression of rubber tubing above	X	X	X	X	X	
Pipettes, 10 ml, graduated, Mohr type, sterile, dispense 10 per can per working space per day. (Resterilize daily to meet need).	X	X	X	X	X	
Pipettes, 1 ml, graduated, Mohr type, sterile, dispense 24 per can per working space per day. (Resterilize daily to meet need).	X	X	X	X	X	
Pipette boxes, sterile, for 1 ml and 10 ml pipettes (sterilize above pipettes in these boxes).	X	X	X	X	X	
Cylinders, 100 ml graduated, sterile, (resterilize daily to meet need).	X	X	X	X	X	
Jars, to receive used pipettes	X	X	X	X	X	
Gas burner, Bunsen or similar laboratory type	X	X	X	X	X	X
Wax pencils, red, suitable for writing on glass	X	X	X	X	X	
Sponge in dilute iodine, to wipe down the desk tops	X	X	X	X	X	
Membrane filters (white, grid marked, sterile,and suitable pore size for microbiological analysis of water)	X	X	X	X	X	
Absorbent pads for nutrient, (47 mm in diameter), sterile, in units of 10 pads per package. Not required if medium contains agar.	X	X	X	X	X	
Petri dishes, disposable, plastic, 50 × 12 mm, sterile	X	X	X	X	X	
Waterbath incubator 44.5 ± 0.2°C				X		
Vegetable crispers, or cake boxes, plastic, with tight fitting covers, for membrane filter incubations	X	X	X		X	
Fluorescent lamp, with extension cord equipped with a simple lens of about 4× magnification	X	X	X	X	X	X
Ring stand, with clamps, utility type	X	X	X	X	X	

Table 1. EQUIPMENT, SUPPLIES AND MEDIA (Cont'd)

	Standard Tests		Nonstandard Tests			
Item	M-Endo Broth	L. E. S. Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Test
M-FC Broth for fecal coliform, dehydrated medium in 25 × 95 mm flat bottomed screw-capped glass vials, 1.11 g per tube, sufficient for 30 ml of culture medium				X		
Rosolic acid, 1% solution, in 0.2N NaOH, in 25 × 150 mm flat bottomed screw-capped tubes, about 5 ml per tube, freshly prepared				X		
M-Enterococcus Agar, dehydrated medium in 25 × 150 mm screw-capped tubes, sufficient for 30 ml, 1.26 g per tube					X	
Dilution bottles, 6-oz, preferable boro-silicate glass, with screw-cap (or rubber stopper protected by paper), each containing 99 ml of sterile phosphate buffered distilled water	X	X	X	X	X	
Electric hot plate surface	X	X	X	X	X	
Beakers, 400 - 600 ml (for water-bath in preparation of membrane filter culture media)	X	X	X	X	X	
Crucible tongs, to be used at electric hot plates, for removal of hot tubes of culture media for boiling waterbath	X	X	X	X	X	

6 Deliver enough culture medium to saturate each absorbent pad,* using a sterile pipette.

Exact quantities cannot be stated because pads and culture containers vary. Sufficient medium should be applied so that when the culture container is tipped, a good-sized drop of culture medium freely drains out of the absorbent pad.

7 Organize supplies and equipment for convenient sample filtration. In training courses, laboratory instructors will suggest useful arrangements; eventually the individual will select a system of bench-top organization most suited to his own needs. The important point in any arrangement is to have all needed equipment and supplies conveniently at hand, in such a pattern as to minimize lost time in useless motions.

8 Lay a sterile membrane filter on the filter holder, grid-side up, centered over the porous part of the filter support plate.

Membrane filters are extremely delicate and easily damaged. For manipulation, the sterile forceps should always grasp the outer part of the filter disk, outside the part of the filter through which the sample passes.

9 Attach the funnel element to the base of the filtration unit.

To aboid damage to the membrane filter, locking forces should only be applied at the locking arrangement. The funnel element never should be turned or twisted while being seated and locked to the lower element of the filter holding unit. Filter holding units featuring a bayonet joint and locking ring to join the upper element to the lower element require special care on the part of the operator. The locking ring should be turned sufficiently to give a snug fit, but should not be tightened excessively.

10 Shake the sample thoroughly.

11 Measure sample into the funnel with vacuum turned off.

The primary objectives here are: 1) accurate measurement of sample; and 2) optimum distribution of colonies on the filter after incubation. To meet these objectives, methods of measurement and dispensation to the filtration assembly are varied with different sample filtration volumes.

a With samples greater than 20 ml, measure the sample with a sterile graduated cylinder and pour it into the funnel. It is important to rinse this graduate with sterile buffered distilled water to preclude the loss of excessive sample volume. This should be poured into the funnel.

b With samples of 10 ml to 20 ml, measure the sample with a sterile 10 ml or 20 ml pipette, and pipette on a dry membrane in the filtration assembly.

c With samples of 2 ml to 10 ml, pour about 20 ml of sterile dilution water into the filtration assembly, then measure the sample into the sterile buffered dilution water with a 10 ml sterile pipette.

d With samples of 0.5 to 2 ml, pour about 20 ml of sterile dilution water into the funnel assembly, then measure the sample into the sterile dilution water in the funnel with a 1 ml or a 2 ml pipette.

e If a sample of less than 0.5 ml is to be filtered, prepare appropriate dilutions in sterile dilution water, and proceed as applicable in item c or d above.

When dilutions of samples are needed, always make the filtrations as soon as possible after dilution of the sample; this never should exceed

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30 minutes. Always shake sample dilutions thoroughly before delivering measured volumes.

12 Turn on the vacuum.

Open the appropriate spring clamp or valve, and filter the sample.

After sample filtration a few droplets of sample usually remain adhered to the funnel walls. Unless these droplets are removed, the bacteria contained in them will be a source of contamination of later samples. (In laboratory practice the funnel unit is not routinely sterilized between successive filtrations of a series). The purpose of the funnel rinse is to flush all droplets of a sample from the funnel walls to the membrane filter. Extensive tests have shown that with proper rinsing technique, bacterial retention on the funnel walls is negligible.

13 Rinse the sample through the filter.

After all the sample has passed through the membrane filter, rinse down the sides of the funnel walls with at least 20 ml of sterile dilution water. Repeat the rinse twice after all the first rinse has passed through the filter. Cut off suction on the filtration assembly.

14 Remove the funnel element of the filter holding unit.

If a ring stand with split ring is used, hang the funnel element on the ring; otherwise, place the inverted funnel element on the inner surface of the wrapping material. This requires care in opening the sterilized package, but it is effective as a protection of the funnel ring from contamination.

15 Take the membrane filter from the filter holder and carefully place it, grid-side up on the medium.

Check that no air bubbles have been trapped between the membrane filter and the underlying absorbent pad or agar. Relay the membrane if necessary.

16 Place in incubator after finishing filtration series.

Invert the containers. The immediate atmosphere of the incubating membrane filter must be at or very near 100% relative humidity.

17 Count colonies which have appeared after incubating for the prescribed time.

A stereoscopic microscope magnifying 10-15 times and careful illumination give best counts.

For reporting results, the computation is:

$$\text{bacteria/100 ml} = \frac{\text{No. colonies counted} \times 100}{\text{Sample volume filtered in ml}}$$

Example:

A total of 36 colonies grew after filtering a 10 ml sample. The number reported is:

$$\frac{\text{36 colonies}}{\text{10 ml}} \times 100 = \text{360 per 100 ml}$$

II MF LABORATORY TESTS FOR COLIFORM GROUP

A Standard Coliform Test (Based on M-Endo Broth MF)

1 Culture medium

a M-Endo Broth MF Difco 0749-02 or the equivalent BBL M-Coliform Broth 01-494

Preparation of Culture Medium (M-Endo Broth) for Standard MF Coliform Test

Yeast extract	1.5	g
Casitone or equivalent	5.0	g
Thiopeptone or equivalent	5.0	g
Tryptose	10.0	g
Lactose	12.5	g
Sodium desoxycholate	0.1	g
Dipotassium phosphate	4.375	g
Monopotassium phosphate	1.375	g
Sodium chloride	5.0	g
Sodium lauryl sulfate	0.05	g
Basic fuchsin (bacteriological)	1.05	g
Sodium sulfite	2.1	g
Distilled water (containing 20.0 ml ethanol)	1000	ml

This medium is available in dehydrated form and it is recommended that the commercially available medium be used in preference to compounding the medium of its individual constituents.

To prepare the medium for use, suspend the dehydrated medium at the rate of 48 grams per liter of water containing ethyl alcohol at the rate of 20 ml per liter.

As a time-saving convenience, it is recommended that the laboratory worker preweigh the dehydrated medium in closed tubes for several days, or even weeks, at one operation.

With this system, a large number of increments of dehydrated medium (e.g., 1.44 grams), sufficient for some convenient (e.g., 30 ml) volume of finished culture medium are weighed and dispensed into screw-capped culture tubes, and stored until needed. Storage should preferably be in a darkened disiccator.

A supply of distilled water containing 20 ml stock ethanol per liter is maintained.

When the medium is to be used, it is reconstituted by adding 30 ml of the distilled water-ethanol mixture per tube of pre-weighed dehydrated culture medium.

b Medium is "sterilized" as directed in I, C.

c Finished medium can be retained up to 96 hours if kept in a cool, dark place. Many workers prefer to reconstitute fresh medium daily.

2 Filtration and incubation procedures are as given in I, D.

Special instructions:

a For counting, use the wide field binocular dissecting microscope, or simple lens. For illumination, use a light source perpendicular to the plane of the membrane filter. A small fluorescent lamp is ideal for the purpose.

b Coliform colonies have a "metallic" surface sheen under reflected light which may cover the entire colony, or it may appear only in the center. Non-coliform colonies range from colorless to pink, but do not have the characteristic sheen.

c Record the colony counts on the data sheet, and compute the coliform count per 100 ml of sample.

B Standard Coliform Tests (Based on L. E. S. Endo Agar)

The distinction of the L. E. S. count is a two hour enrichment incubation on LST broth. M-Endo L. E. S. medium is used as agar rather than the broth.

1 Preparation of culture medium (L. E. S. Endo Agar) for L. E. S. coliform test

a Formula from McCarthy, Delaney, and Grasso (2)

Bacto-Yeast Extract	1.2 g
Bacto-Casitone	3.7 g
Bacto-Thiopeptone	3.7 g
Bacto-Tryptose	7.5 g
Bacto-Lactose	9.4 g
Dipotassium phosphate	3.3 g
Monopotassium phosphate	1.0 g
Sodium chloride	3.7 g
Sodium desoxycholate	0.1 g
Sodium lauryl sulfate	0.05 g
Sodium sulfite	1.6 g
Bacto-Basic fuchsin	0.8 g
Agar	15 g
Distilled water (containing 20 ml ethyl alcohol)	1000 ml

b To rehydrate the medium, suspend 51 grams in the water-ethyl alcohol solution.

c Medium is "sterilized" as directed in I, C.

d Pour 4-6 ml of freshly prepared Agar into the smaller half of the container. Allow the medium to cool and solidify.

2 Procedures for filtration and incubation

a Lay out the culture dishes in a row or series of rows as usual. Place these with the upper (lid) or top side down.

b Place one sterile absorbent pad in the larger half of each container (lid). Use sterile forceps for all manipulations of the pads. (Agar occupies smaller half or bottom).

c Using a sterile pipette, deliver enough single strength lauryl sulfate tryptose broth to saturate the pad only. Excess interferes.

d Follow general procedures for filtering in I, D. Place filters on pad with lauryl sulfate tryptose broth.

e Upon completion of the filtrations, invert the culture containers and incubate at 35°C for 1 1/2 to 2 hours.

3 2-hour procedures

a Transfer the membrane filter from the enrichment pad in the upper half to the agar medium in the lower half of the container. Carefully roll the membrane onto the agar surface to avoid trapping air bubbles beneath the membrane.

b Removal of the used absorbent pad is optional.

c The container is inverted and incubated 22 hours ± 2 hours ± 0.5°C.

4 Counting procedures are as in I, D.

5 L. E. S. Endo Agar may be used as a single-stage medium (no enrichment step) in the same manner as M-Endo Broth, MF.

C Delayed Incubation Coliform Test

This technique is applicable in situations where there is an excessive delay between sample collection and plating. The procedure is unnecessary when the interval between sample collection and plating is within acceptable limits.

1 Preparation of culture media for delayed incubation coliform test

a Preservative media M-Endo Broth base

To 30 ml of M-Endo Broth MF prepared in accordance with directions in II, A, 1 of this outline, add 1.0 ml of a sterile 12% aqueous solution of sodium benzoate.

L.E.S. MF Holding Medium-Coliform: Dissolve 12.7 grams in 1 liter of distilled water. No heating is necessary. Final pH 7.1 ± 0.1. This medium contains sodium benzoate.

b Growth media

M-Endo Broth MF is used, prepared as described in II, A, 1 earlier in this outline. Alternately, L.E.S. Endo Medium may be used.

2 General filtration followed is in I, D.

Special procedures are:

a Transfer the membrane filter from the filtration apparatus to a pad saturated with benzoated M-Endo Broth.

b Close the culture dishes and hold in a container at ambient temperature. This may be mailed or transported to a central laboratory. The mailing or transporting tube should contain accurate transmittal data sheets which correspond to properly labeled dishes.

Transportation time, in the case of mailed containers, should not exceed three days to the time of reception by the testing laboratory.

c On receipt in the central laboratory, unpack mailing carton, and lay out the culture containers on the laboratory bench.

d Remove the tops from the culture containers. Using sterile forceps, remove each membrane and its absorbent pad to the other half of the culture container.

e With a sterile pipette or sterile absorbent pad, remove preservative medium from the culture container.

f Place a sterile absorbent pad in each culture container, and deliver enough freshly prepared M-Endo Broth to saturate each pad.

g Using sterile forceps, transfer the membrane to the new absorbent pad containing M-Endo Broth. Place the membrane carefully to avoid entrapment of air between the membrane and the underlying absorbent pad. Discard the absorbent pad containing preservative medium.

h After incubation of 20 ± 2 hours at 35°C, count colonies as in the above section A, 2.

i If L.E.S. Endo Agar is used, the steps beginning with (e) above are omitted; and the membrane filter is removed from the preservative medium and transferred to a fresh culture container with L.E.S. Endo Agar, incubated, and colonies counted in the usual way.

D Verified Membrane Filter Coliform Test

This procedure applies to identification of colonies growing on Endo-type media used for determination of total coliform counts. Isolates from these colonies are studied for gas production from lactose and typical coliform morphology. In effect, the procedure corresponds with the Completed Test stage of the multiple fermentation tube test for coliforms.

Procedure:

1 Select a membrane filter bearing several well-isolated coliform-type colonies.

2 Using sterile technique, pick all colonies in a selected area with the inoculation needle, making transfers into tubes of phenol red lactose broth (or lauryl sulfate tryptose lactose

broth). Using an appropriate data sheet record the interpretation of each colony, using, for instance, "C" for colonies having the typical color and sheen of coliforms; "NC" for colonies not conforming to coliform colony appearance on Endotype media.

3 Incubate the broth tubes at 35° C ± 0.5°C.

4 At 24 hours:

a Read and record the results from the lactose broth fermentation tubes. The following code is suggested:

Code	
O	No indication of acid or gas production, either with or without evidence of growth.
A	Evidence of acid but not gas (applies only when a pH indicator is included in the broth medium)
G	Growth with production of gas. If pH indicator is used, use symbol AG to show evidence of acid. Gas in any quantity is a positive test.

b Tubes not showing gas production are returned to the 35° C incubator.

c Gas-positive tubes are transferred as follows:

1) Prepare a streak inoculation on EMB agar for colony isolation, and using the same culture.

2) Inoculate a nutrient agar slant.

3) Incubate the EMB agar plates and slants at 35° C ± 0.5°C.

5 At 48 hours:

a Read and record results of lactose broth tubes which were negative at 24 hours and were returned for further incubation.

b Gas-positive cultures are subjected to further transfers as in 4c. Gas-negative cultures are discarded without further study; they are coliform- negative.

c Examine the cultures transferred to EMB agar plates and to nutrient agar slants, as follows:

1) Examine the EMB agar plate for evidence of purity of culture; if the culture represents more than one colony type, discard the nutrient agar culture and reisolate each of the representative colonial types on the EMB plate and resume as with 4c for each isolation. If purity of culture appears evident, continue with c (2) below.

2) Prepare a smear and Gram stain from each nutrient agar slant culture. The Gram stain should be made on a culture not more than 24 hours old. Examine under oil immersion for typical coliform morphology, and record results.

6 At 72 hours:

Perform procedures described in 5c above, and record results.

7 Coliform colonies are considered verified if the procedures demonstrate a pure culture of bacteria which are gram negative nonspore-forming rods and produce gas from lactose at 35° C within 48 hours.

E Fecal Coliform Count (Based on M-FC Broth Base)

The count depends upon growth on a special medium at 44.5 ± 0.2°C.

1 Preparation of Culture Medium (M-FC Broth Base) for Fecal Coliform Count

a Composition

Tryptose	10.0 g
Proteose Peptone No. 3	5.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose	12.5 g
Bile salts No. 3	1.5 g
Rosolic acid* (Allied Chemical)	10.0 ml
Aniline blue (Allied Chemical)	0.1 g
Distilled water	1000 ml

b To prepare the medium dissolve 37.1 grams in a liter of distilled water which contains 10 ml of 1% rosolic acid (prepared in 0.2 N NaOH).

Fresh solutions of rosolic acid give best results. Discard solutions which have changed from dark red to orange.

c To sterilize, heat to boiling as directed in I, C.

d Prepared medium may be retained up to 4 days in the dark at 2-8° C.

2 Special supplies

Small water proof plastic sacks capable of being sealed against water with capacity of 3 to 6 culture containers.

3 Filtration procedures are as given in I, D.

4 Elevated temperature incubation

a Place fecal coliform count membranes at 44.5 ± 0.2°C as rapidly as possible.

Filter membranes for fecal coliform counts consecutively and immediately place them in their culture containers. Insert as many as six culture containers all oriented in the same way (i.e., all grid sides facing the same direction) into the sacks and seal. Tear off the perforated top, grasp the side wires, and twirl the sack to roll the open end inside the folds of sack. Then submerge the sacks with culture containers inverted beneath the surface of a 44.5 ± 0.2°C waterbath.

b Incubate for 22 ± 2 hours.

5 Counting procedures

Examine and count colonies as follows:

a Use a wide field binocular dissecting microscope with 5 - 10X magnification.

b Low angle lighting from the side is advantageous.

c Fecal coliform colonies are blue, generally 1-3 mm in diameter.

d Record the colony counts on the data sheet, and report the fecal coliform count per 100 ml of sample. (I, D, 17 illustrates method)

III TESTS FOR FECAL STREPTOCOCCAL GROUP-MEMBRANE FILTER METHOD

A 48 hour incubation period on a choice of two different media, giving high selectivity for fecal streptococci, are the distinctive features of the tests.

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*Prepare 1% solution of rosolic acid in 0.2 N NaOH. This dye is practically insoluble in water.

A Test for Members of Fecal Streptococcal (Tentative, Standard Methods) M-Enterococcus Agar Medium

1 Preparation of the culture medium

a Formula (The Difco formula is shown, but equivalent constituents from other sources are equally acceptable).

Bacto tryptose	20.0 g
Bacto yeast extract	5.0 g
Bacto dextrose	2.0 g
Dipotassium phosphate	4.0 g
Sodium Azide	0.4 g
Bacto agar	10.0 g
2, 3, 5, Triphenyl tetrazolium chloride	0.1 g

b The medium is prepared by rehydration at the rate of 42 grams per 1000 ml of distilled water. It is recommended that the medium in dehydrated form be preweighed and dispensed into culture containers (about 25 × 150 mm) in quantities sufficient for preparation of 30 ml of culture medium (1.26 g per tube).

c Follow I, C, for "sterilizing" medium and dispense while hot into culture containers. Allow plates to harden before use.

2 List of apparatus, materials, as given in Table I.

3 Procedure, in general, as given in I.

Special instructions

a Incubate for 48 hours, inverted, with 100% relative humidity, after filtrations are completed. If the entire incubator does not have saturated humidity, acceptable conditions can be secured by placing the cultures in a tightly closed container with wet paper, towels, or other moist material.

b After incubation, remove the cultures from the incubator, and count all colonies under wide field binocular dissecting microscope with magnification set at 10X or 20X. Fecal streptococcus colonies are 0.5 - 2 mm in diameter, and flat to raised smooth, and vary from pale pink to dark red in color.

c Report enterococcus count per 100 ml of sample. This is conveniently computed:

No. fecal streptococci per 100 ml =

$$\frac{\text{No. fecal streptococcus colonies counted}}{\text{Sample filtration volume in ml}} \times 100$$

B Test for Members of Fecal Streptococcal Group based on KF-Agar

1 Preparation of the culture medium

a Formula: (The dehydrated formula of Bacto 0496 is shown, but equivalent constituents from other sources are acceptable). Formula is in grams per liter of reconstituted medium.

Bacto proteose peptone #3	10.0 g
Bacto yeast extract	10.0 g
Sodium chloride (reagent grade)	5.0 g
Sodium glycerphosphate	10.0 g
Maltose (CP)	20.0 g
Lactose (CP)	1.0 g
Sodium azide (Eastman)	0.4 g
Sodium carbonate (Na_2CO_3 reagent grade)	0.636 g
Brom cresol purple (water soluble)	0.015 g
Bacto agar	20.0 g

b Reagent

2, 3, 5-Triphenyl tetrazolium chloride reagent (TPTC)

This reagent is prepared by making a 1% aqueous solution of the above chemical passing it through a Seitz filter or membrane filter. It can be kept in the refrigerator in a screw-capped tube until used.

c The dehydrated medium described above is prepared for laboratory use as follows:

Suspend 7.64 grams of the dehydrated medium in 100 ml of distilled water in a flask with an aluminum foil cover.

Place the flask in a boiling waterbath, melt the dehydrated medium, and leave in the boiling waterbath an addional 5 minutes.

Cool the medium to 50°-60° C, add 1.0 ml of the TPTC reagent, and mix.

For membrane filter studies, pour 5-8 ml in each 50 mm glass or plastic culture dish or enough to make a layer approximately 1/8" thick. Be sure to pour plates before agar cools and solidifies.

For plate counts, pour as for standard agar plate counts.

NOTE: Plastic dishes containing media may be stored in a dark, cool place up to 30 days without change in productivity of the medium, provided that no dehydration occurs. Plastic dishes may be incubated in an ordinary air incubator. Glass dishes must be incubated in an atmosphere with saturated humidity.

2 Apparatus, and materials as given in Table 1.

3 General procedure is as given in I.

Special instructions

a Incubate 48 hours, inverted with 100% relative humidity after filtration.

b After incubation, remove the cultures from the incubator, and count colonies under wide field binocular dissecting microscope, with magnification set at 10X or 20X. Fecal streptococcus colonies are pale pink to dark wine-color. In size they range from barely visible to approximately 2mm in diameter. Colorless colonies are not counted.

c Report fecal streptococcus count per 100 ml of sample. This is computed as follows:

No. fecal streptococci per 100 ml =

$$\frac{\text{No. fecal streptococcus colonies}}{\text{Sample filtration volume in ml}} \times 100$$

C Verification of Streptococcus Colonies

1 Verification of colony identification may be required in waters containing large numbers of Micrococcus organisms. This has been noted particularly with bathing waters, but the problem is by no means limited to such waters.

2 A verification procedure is described in "Standard Methods for the Examination of Water and Wastewater," 13th ed. (1971). The worker should use this reference for the step-by-step procedure.

IV PROCEDURES FOR USE OF MEMBRANE FILTER FIELD UNITS

A Culture Media

1 The standard coliform media used with laboratory tests are used.

2 To simplify field operations, it is suggested that the medium be sent to the field, preweighed, in vials or capped culture tubes. The medium then requires only the addition of a suitable volume of distilled water-ethanol prior to sterilization.

3 Sterilization procedures in the field are the same as for laboratory methods.

4 Laboratory preparation of the media, ready for use, would be permissible provided that the required limitations on time and conditions of storage are met.

B Operation of the Sabro Field Unit

1 Equipment and materials

Sabro field unit

Membrane filters

Absorbent pads for nutrient

Culture containers

M-Endo Broth MF or L.E.S. Endo Medium

Provision for heating water (optional)

Source of electricity

2 Procedure (Based on M-Endo Broth MF)

a Connect the electric cord to the power source and to Sabro field unit. After about 15 minutes check the temperature in the incubator drawer. The required temperature is 35° C (95° F). If the temperature is too low it can be increased by turning the thermostat adjustment screw counterclockwise. This screw is located at the front on the recessed divider between the two incubator drawers. To lower temperature, turn the adjustment screw clockwise.

b Review the supply of expendable materials to be used with the unit and secure replacements as needed (culture containers, medium, membrane filters, absorbent pads for nutrient, fuel, etc.).

c Sterilize the funnel unit by one of the following procedures.

1) Immerse the equipment 2 minutes in boiling water. The temperature should be at least 78° C (170° F).

2) Flame-sterilize membrane filter holder inside and both ends of funnel (suggested by manufacturer).

d Lay in a row all the culture containers to be used in the filtration series, and number the containers to correspond with numbers of the data sheet.

e Place one sterile absorbent pad in each culture container. Use sterile forceps for all manipulation of absorbent pads and filters.

f Using sterile pipette deliver enough culture medium to saturate each absorbent pad. The amount of culture medium required is approximately 2 ml, but cannot be precisely stated. Sufficient medium should be applied, that when the culture container is tipped, a good-sized drop of culture medium freely drains out of the absorbent pad.

g Using sterile forceps, place a membrane filter, grid-side up, on the MF receptacle of the funnel unit. Place the funnel portion over the membrane, and clamp the unit with the spring clamp provided with the portable kit.

h Pour the water sample into the funnel using a sterile pipette or graduate.

i Connect the tubing of the vacuum pump to the receptable on the base of the filter unit and draw the sample through the membrane.

filter, rinse down the sides of the funnel walls with at least 20 ml of sterile dilution water. Repeat the rinse twice after all the first rinse has passed through the filter.

j Disassemble the funnel unit and with sterile forceps transfer the membrane filter grid-side up to the appropriate culture container. The membrane should be "rolled on" the absorbent pad containing culture medium, to prevent entrapment of air between the pad and the membrane filter.

k Repeat steps g - j for additional filtrations of the same or different sampling volumes for the water being tested.

l After completion of filtration, place the culture container in an inverted position (with membrane position grid-side down) in the incubator drawers.

m After completion of the last filtration from any one sample, resterilize the funnel unit by one of the procedures described in instruction 2c.

n Allow the cultures to incubate 20 - 24 hours.

o Remove the cultures from the incubator and count coliform colonies.

C Operation of Millipore Water Testing Kit, Bacteriological

1 Supporting supplies and equipment are the same as for the laboratory procedures.

2 Set the incubator voltage selector switch to the voltage of the available supply, turn on the unit and adjust as necessary to establish operating incubator temperature at 35 ± 0.5°C.

3 Sterilize the funnel unit assembly by exposure to formaldehyde or by immersion in boiling water. If a laboratory autoclave is available, this is preferred.

Formaldehyde is produced by soaking an asbestos ring (in the funnel base) with methanol, igniting, and after a few seconds of burning, closing the unit by placing the stainless steel flask over the funnel and base. This results in incomplete combustion of the methanol, whereby formaldehyde is produced. Leave the unit closed for 15 minutes to allow adequate exposure to formaldehyde.

4 Filtration and incubation procedures correspond with laboratory methods.

5 The unit is supplied with a booklet containing detailed step-by-step operational procedures. The worker using the equipment should become completely versed in its contents and application.

D Counting of Colonies on Membrane Filters

1 Equipment and materials

Membrane filter cultures to be examined

Illumination source

Simple lens, 2X to 6X magnification

Hand tally (optional)

2 Procedure

a Remove the cultures from the incubator and arrange them in numerical sequence.

b Set up illumination source as that light will originate from an area perpendicular to the plane of membrane filters being examined. A small fluorescent lamp is ideal for the purpose. It is highly desirable that a simple lens be attached to the light source.

c Examine results. Count all coliform and noncoliform colonies. Coliform colonies have a "metallic" surface sheen under reflected light, which may cover the entire colony or may appear only on the center.

Noncoliform colonies range from colorless to pink or red, but do not have the characteristic "metallic" sheen.

d Enter the colony counts in the data sheets.

e Enter the coliform count per 100 ml of sample for each membrane having a countable number of coliform colonies. Computation is as follows:

No. coliform per 100 ml =

$$\frac{\text{No. coliform colonies on MF}}{\text{No. milliliters sample filtered}} \times 100$$

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WPCF. 12th Edition. 1965.

2 McCarthy, J.A., Delaney, J.E. and Grasso, R.J. Measuring Coliforms in Water. Water and Sewage Works. 1961: R-426-31. 1961.

This outline was prepared by H. L. Jeter, Director, National Training Center, EPA, WPO, Cincinnati, OH 45268.

Descriptors: Biological Membranes, Coliforms, Fecal Coliforms, Fecal Streptococci, Filters, Indicator Bacteria, Laboratory Equipment, Laboratory Tests, Membranes, Microbiology, Water Analysis

OXYGEN DEMAND ANALYSES

I INTRODUCTION

A Oxygen demand analyses are basic water pollution control tools. Treatment plant design is largely based on empirical evidence expressed in terms of 5-day biochemical oxygen demand (BOD). First-stage BOD, second-stage BOD, 20-day BOD, and ultimate BOD are familiar terms to oxygen resource analysts.

Modern research in treatment technology employs more exotic analyses such as chemical oxygen demand (COD) and various methods of estimating theoretical oxygen demand (TOD). It is perhaps fair to say that more pollution control decisions are based upon oxygen demand data than upon other analytical results.

B Yet interpretation of these data often seems an insurmountably difficult task. There should be no mystery about such interpretation; oxygen demand analyses are powerful tools which must be used with informed skill. Those problems which arise are more related to complexity of the environmental situation than to inherent analytical errors.

II IMPLICATIONS

A Positive oxygen demand results imply one or more of the following:

1 The presence of a compound which will exert an oxygen demand in the natural environment

2 The presence of organic matter

B These statements are not necessarily both true at the same time, i.e., there are compounds (e.g. NH_3) which will exert oxygen demand in nature but are not chemically organic. Likewise there are organic compounds which, under favorable laboratory or environmental conditions, will not exert a measurable oxygen demand.

C Nor is the converse of either statement necessarily true. The presence of a suitable organic or inorganic compound does not guarantee a positive oxygen demand, especially under laboratory conditions.

D It is, perhaps, better to say that positive oxygen demand values indicate the presence of a compound which may potentially exert a natural O_2 demand or may potentially be organic in nature. Generally one or both implications proves true, but the exceptions are often crucial.

III COMMUNICATIONS BARRIERS

Oxygen demand terminology tends to present a communications barrier. To a chemist COD may represent the result of a prescribed analytical procedure; to an engineer it may represent a rather abstract concept of the amount of oxygen utilized in a purely chemical reaction. Of course this sort of conflict can never be decided; it is analagous to the philosophical trap of deciding whether there is noise when a tree falls in the forest in the absence of an observer. The practical solution is that each practitioner develop his knowledge of COD, BOD, etc. to include several concepts. During discussion or writing, the sense in which an oxygen demand term is used must be clearly defined.

IV DEFINITIONS

A The THEORETICAL OXYGEN DEMAND of a waste is the amount of oxygen required for oxidation of the components of that waste to their highest attainable oxidation states. Theoretical oxygen demand would be attained only by perfect combustion at elevated temperature in the presence of excess oxygen.

For example, if a waste were composed of C^{+4}, NO_2^-, and S, the theoretical oxygen demand of that waste would be the amount of oxygen required to convert these components to CO_2, NO_3^-, and $SO_4^=$.

TOD represents the standard to which all other oxygen demand methods may be compared. It is a unique, abstract value which has never been actually measured.

B BIOCHEMICAL OXYGEN DEMAND is defined as the amount of oxygen required for biochemical oxidation of organic and/or inorganic matter. The ULTIMATE biochemical oxygen demand would be exerted if the biochemical reactions were allowed to progress to completion. the ULTIMATE FIRST STAGE demand would be exerted upon completion of oxidation of carbonaceous compounds; 5-day biochemical oxygen demand is the amount of oxygen utilized during five days of biochemical oxidation. Depending upon sample history these five days may represent any 5-day segment of the BOD curve. The relationship of the various classes of BOD is shown in Figure 1.

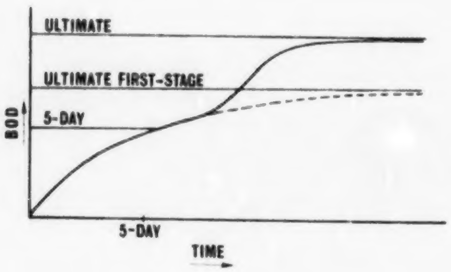


Figure 1. CLASSICAL BOD PROGRESSION

The second step of the BOD curve may be caused by nitrification and/or low-rate oxidation of resistant compounds. The shape of the curve is not a valid indicator of the type of oxidation occurring. ULTIMATE BOD and ULTIMATE FIRST-STAGE BOD are unique, abstract values which can never be reached. If one presupposes an infinite variety of organisms, infinite biological adaptability, and infinite reaction time, ULTIMATE BOD must equal THEORETICAL OXYGEN DEMAND.

C CHEMICAL OXYGEN DEMAND is the oxygen equivalent of the amount of active agent consumed during oxidation of a waste by a strong chemical oxidant, other than oxygen. COD varies according to the compound, the reagents, and the procedures employed during analysis. Standard Methods (1) specifies a dichromate oxidation in 50% sulfuric acid at approximately 145°C; an older method - called the OXYGEN CONSUMED test - employs potassium permanganate as an oxidizing agent. If performed on the same waste, the COD and OXYGEN CONSUMED analyses would produce different numerical results.

D IMMEDIATE OXYGEN DEMAND is that oxygen demand exerted spontaneously upon contact of a waste with oxygen. It may include oxygen utilized in reaction with ferrous iron, sulfites, sulfides, sulfhydryl groups, or other reducing agents which will react within 15 minutes of contact.

III TEST PROCEDURES AND INTERPRETATIONS

A Dissolved Oxygen (DO) Determinations

Quantitative measurements of dissolved oxygen levels are made by chemical or electrometric methods. Various ions and compounds interfere with the chemical determination of DO; when using chemical methods, one should be alert to their presence. The Azide Modification of the basic Winkler Method (1) is the chemical procedure most commonly used; other modifications have been developed to correct for various interferences.

Electrometric methods have recently become more popular; such methods may produce a continuous oxygen record, are easily adapted to remote telemetry, and tend to overcome certain interferences with the Winkler method.

Dissolved oxygen data may:

1 Serve as an indirect measurement of photosynthetic activity;

2 Indirectly indicate the progress of biological stabilization;

3 Form the chemical bases for the BOD and IOD tests.

B Biochemical Oxygen Demand Test (BOD)

1 Indicates oxygen demand potential of a waste when exposed to a biological environment.

2 Indirectly used as a measure of available material (specific analyses for a myriad of organic compounds found in domestic and industrial wastes would be time-consuming and pointless for most cases).

3 Gives an idea of the rate at which biodegradable organic material is being assimilated.

4 Long-term BOD is invariably less than the theoretical oxygen demand because:

   a Chemical compounds differ with respect to availability as a biological nutrient.

   b Biological action tends to convert nutrients to other substances of decreasing availability.

   c Adapted organisms may not be present.

   d The environment is not always favorable for optimum biological action.

5 At the discretion of the analyst reported BOD test results may or may not include immediate oxygen demand (IOD). When results are reported or analyzed, inclusion or non-inclusion should be specified.

C Chemical Oxygen Demand Test (COD)

1 COD indicates the presence of organic and/or inorganic material susceptible to chemical oxidation. This material may also be biologically oxidizable.

2 For certain wastes, a definite BOD/COD relationship may exist; this is true with domestic sewage, for which COD may approximate ultimate first-stage BOD.

   a High COD/BOD ratio on an organic waste which, from all known characteristics should be biodegradable, may indicate the presence of toxic agents in the waste.

   b A high COD/BOD ratio may also indicate that the waste is resistant to biodegradation.

3 Although COD usually exceeds BOD, the reverse may be true with certain wastes, notably cellulose. In such cases, COD is not a reliable measure of deoxygenation potential. Ideally, neither COD nor BOD should be used as the sole measure of deoxygenation potential.

V SUMMARY

Oxygen demand analyses are key pollution control tools. Positive results indicate potential oxygen demand in nature and/or the potential presence of organic matter. Generally the implications are true, but there may be important exceptions. Valid use of such data requires careful and knowledgeable interpretation.

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater, AWWA, APHA, WPCF, 12th Edition. (1965)

2 Sawyer, C. N. Chemistry for Sanitary Engineers, McGraw-Hill, New York. (1960)

This outline was prepared by F.P. Nixon, former Acting Regional Training Officer, Northeast Regional Training Center, OWP, Hudson-Delaware Basins Office, Edison, NJ 08817.

Descriptors: Biochemical Oxygen Demand, Chemical Oxygen Demand, Dissolved Oxygen, Oxygen Demand

EFFECT OF SOME VARIABLES ON THE BOD TEST

I TIME

A The common equation $y_t = L\ (1 - 10^{-kt})$ for BOD relationship indicates time as a variable. The rate coefficient (k_1) indicates that a specific percentage of material initially present (oxygen) will be used during a given time unit. Each successive unit of time has less reactant present initially than the preceding interval, hence a definite precentage decrease results in successively smaller amounts of reactant use per unit of lapsed time. Increasing k_1 results in a larger percentage oxygen use per unit of time and also increases the change in reactant mass among successive time intervals.

B Adney's work for the British Royal Commission cited 5 days passage time from source to the ocean as maximum for English streams. The 8th Report (1909) largely established BOD philosophy including the 5-day interval. At 5 days, initial lags generally have terminated and a substantial fraction of the long-term oxygen demand has been exerted. If only one time interval can be used, 7 days permits better scheduling. Any one time interval is "a" fraction of the total oxygen requirement; this is a poor reference point if we do not know how it arrived. For example, the percentage of oxidizable material stabilized in terms of oxygen use at various rate factors are:

k_1 $(\log_{10})$	% oxidized in 5 days	K_1 $(\log_e)$
0.05	42%	0.11
0.10	67%	0.23
0.15	84%	0.34
0.25	94%	0.57
0.50	99+%	1.15

This range $(K_1 = 2.3\ k_1)$ is commonly encountered in wastewater stabilization with the higher rates characteristic of fresh oxidizable material that is readily converted.

The lower coefficients are characteristic of cell mass at later stages of oxidation and of low-rate reactants in general.

C The oxygen utilization at specified intervals of time are required to estimate k_1, and L, the estimate of oxygen use at infinite time. It is common to observe results at equal intervals of time but this is not essential as long as the time intervals are accurately known. The initial time periods are critical as an error of a few hours in time represents a relatively large change in reactant mass in a system at maximum instability. Unequal time periods can be plotted to define the curve from which any given intervals can be selected as desired.

D Increasing impoundment of surface water provides more time for stabilization of relatively inert soluble or suspended pollutants and for organism adaptation to the situation or pollutants. Long term BOD's are essential to indicate changes in the pattern of oxygen demand vs. time. It may be expected that one or more plateaus will be evident in the BOD curve followed by a temporary rise in rate during second stage oxidation or thereafter. Anaerobiosis may cause a rise in rate coefficient after aerobic conditions are re-established. Eventually k_1 stabilizes at very low values.

1 Rate coefficients tend to be difficult to interpret during long term BOD's because of progressive changes and other factors.

a The relative error of the DO test may be a large fraction of the incremental DO change during low rate periods.

b Cell mass may agglomerate under quiescent test conditions and decrease nutrient availability.

c It is not likely that recycled nutrients under aerobic test conditions will have as much effect as recycle from anaerobic benthic deposits in a stream.

2 The BOD result tends to underestimate deoxygenation relative to surface water behavior because of interchanges, turbulence, biota, and boundary effects. Reseeding does not occur in a sealed bottle but reseeding is inevitable in a stream or treatment unit.

II TEMPERATURE

A Effect on Oxidation Rate

Temperature is one of the important controlling factors in any biological system. In the BOD reaction, changes in temperature produce acceleration or depression of the rate of oxidation. Figure 1 shows the changes in the value of k at temperatures from 0 - 25°C on a common wastewater.

B Test Temperature

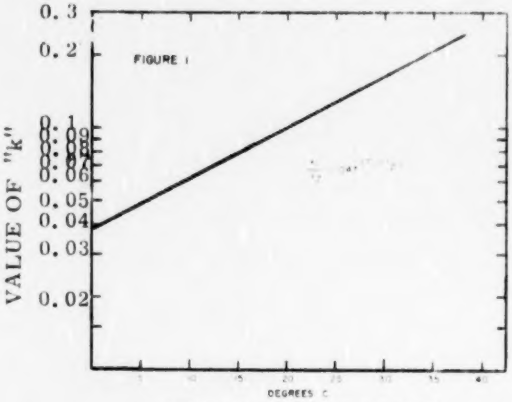
In the BOD test procedure an arbitrary temperature is usually selected for convenience even though a wide temperature range exists under natural conditions.

Incubation of the test containers at 20°C for the whole period is now accepted practice in the U.S.; 18.5°C is preferred in England. Camp (ASCE, SA5 91:1, Oct. 65) recommended light and dark bottle immersion in the stream.

C Temperature Correction

When it is necessary to calculate the rate of oxidation at a temperature other than 20°, the following relationship may be used:

$$\frac{k_1}{k_2} = \theta^{(T_1 - T_2)}$$



VARIATION IN "k" WITH CHANGE IN TEMPERATURE

where:

k_1 = rate coefficient at temperature T_1

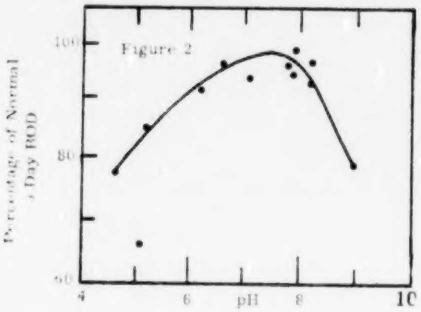
k_2 = rate at coefficient at temperature T_2

θ = temperature coefficient, for which Streeter and Phelps obtained the value 1.047. θ changes with temperature; it appears to be higher in the range of 5-15°C than in the range of 30 to 40°C. The value given refers to 15-30°.

The cited temperature coefficient appears reasonable for household wastes. It may not apply for other wastes where developing or seed organisms may not tolerate temperature changes as readily. A given temperature coefficient should be checked for applicability under specified conditions.

III pH

A The organisms involved in biochemical conversions apparently have an optimum response near a pH of 7.0 providing other environmental factors are favorable; a pH range of about 6.5 to 8.3 apparently is acceptable (Figure 2). Reactivity is likely to be significantly lower on both sides of the acceptable pH range but microbial adaptation may extend the limits appreciably. For example, trickling filters have operated with better than 50% treatment efficiency at pH 3 and 10 after adaptation.



B Adjustment of Concentrated Samples

When wastes are more acid than pH 6.5 or more alkaline than pH 8.3, adjustment to pH 7.2 is advisable before reliable BOD values can be obtained.

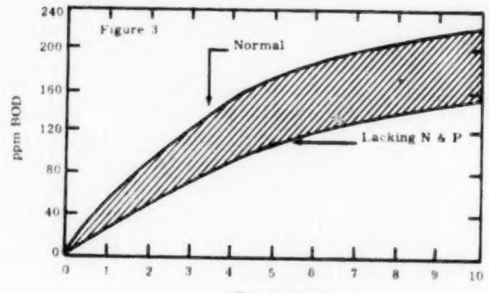
C Dilution Samples

Standard dilution water is buffered at pH 7.2. Sample-dilution water mixtures should be checked to make sure that the sample buffer capacity does not exceed the capacity of the dilution water for pH adjustment.

IV ESSENTIAL MINERAL NUTRIENTS

A Importance

In 1932 Butterfield reported on the role of certain minerals in the biochemical oxidation of sewage and concluded that deficient minerals often upset metabolic response. In addition, he found that inadequate nitrogen and/or phosphorus was a common cause of low BOD results in industrial wastewaters. (Figure 3)



Effect of Mineral Nutrients on BOD

B Standard Methods Dilution Water

The dilution water specified for the BOD test approximates USGS estimates for an average U.S. mineral content of surface water except for added phosphate buffer. It is assumed to provide essential mineral nutrients for most wastewaters but cannot be expected to meet requirements for grossly deficient wastewater nutrients both mineral and organic. Ruchhoft (S.W.J. 13:669, 1941) summarized committee action leading to the present dilution water.

C Other Dilution Considerations

There is a trend toward the use of receiving water, storage-stabilized if necessary, to evaluate waste behavior. It is advisable to minimize dilution and consider the nutrient level likely in the receiving water as most valid. Any change in the environment, such as dilution, upsets the microbial balance and requires adaptive changes.

V MICROBIOLOGICAL POPULATION

A Need for Complex Flora and Fauna

Butterfield, Purdy, and Theriault (Pub. Health Rep. 393, 1931) demonstrated that an isolated species of organisms was not as effective in biological stabilization as a variety of species. Figure 4 summarizes some of their data. Bhatta and Gaudy (ASCE, SA3, 91:63, June 1965) reinvestigated this factor. Many studies have emphasized the need for a mixed biota in the BOD test. It appears that bacteria are capable of varied activities, but all species are not capable of synthesizing all required nutrients. Certain bacterial species may be capable of producing enzymes, amino acids, or growth factors needed for their use and by other species for optimum performance. It has been shown that oxygen demand becomes minimal when some limit of bacterial population has been reached. Predation prevents such an approach to maximum numbers and maintains a continuing bacterial growth and recycle of nutrients among a mixed population. The net effect is a symbiotic relation among mixed organisms tending to enhance the rate of stabilization or utilization of oxygen as in the BOD test.

B Organism Adaptation

1 Early investigations in relation to the BOD test considered domestic wastewaters primarily. The saprophytic organisms involved in stabilization either were present in adequate numbers or quickly multiplied to attain effective populations.

2 The period of adjustment required to shift enzyme production needed to utilize an energy source different from that previously utilized or to shift population variety from that favored by one food to that favored by another food is considered an adaptation period. Dilution, temperature, oxygen tension, pH, nutrient type, inhibitory substances, light and other changes all are common inducements for microbial adaptation. Mutation of organisms may be encountered during adaptation but usually is not a factor.

3 The developments in industry and technology have resulted in discharge of new and more varied wastewater constituents. Microorganisms may adapt themselves to the use of a new substance as an energy source providing the energy and environment are favorable. The receiving stream usually shows development of an adapted microbiota for a new or different discharge constituent within hours, days or weeks after fairly regular discharge. The time for adaptation depends on the nature of the constituent, available energy, tolerance of the organisms, and environmental conditions.

C Seeding

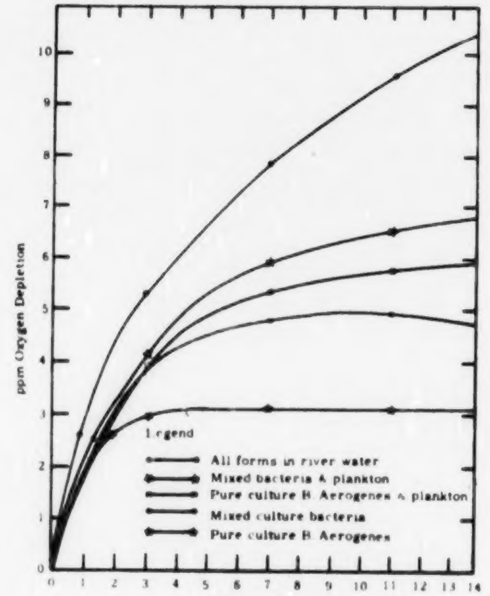
The amount of seed and its selection must be determined experimentally. The most effective inoculant would be that which would produce the maximum BOD response with minimum lag period and negligible seed demand. This would mean some maximum population adapted to feed and conditions at a minimum equilibrium energy nutrient supply.

1 Figure 5 indicates corrected BOD progression on a synthetic feed with river water and stale sewage inoculants at several concentrations. The river water resulted in higher BOD with negligible lag and seed correction. The seed correction at 20% concentration of inoculant was less than 0.3 mg. DO/l at 5 days. It would be possible to use this river water as a diluent without excessive oxygen loss to produce more valid BOD progression for that receiving water. The lower wastewater inoculant concentration resulted in a definite BOD lag. Higher wastewater concentrations produced comparable BOD progression earlier but resulted in high seed corrections and lowered availability of dissolved oxygen for the sample.

2 A good secondary treated effluent produced results similar to river water inoculation with higher seed corrections per increment of applied inoculant. Soil suspensions also are very effective sources of seed organisms with minor seed corrections if they are reasonably stabilized surface soils.

3 It appears that the BOD progression most nearly indicating receiving water oxidation would be one based upon receiving water dilution or inoculated with organisms from it.

4 A new or unusual wastewater may require adapted organisms not present in sufficient numbers in the receiving water. Development of an adapted seed from soil suspensions, plant effluents or receiving water may be necessary to evaluate oxidation potential in a plant or receiving water at some future time. Enrichment culture technique is beneficial where small concentrations of the test wastewater are applied regularly with increases in wastewater concentrations as BOD or respiration activity indicates increasing tolerance and oxidation of the test waste. Both time and concentration limits are useful to characterize the wastewater and its acceptability for biological stabilization.



Effect of Biological Forms on Oxygen Depletion
Figure 4

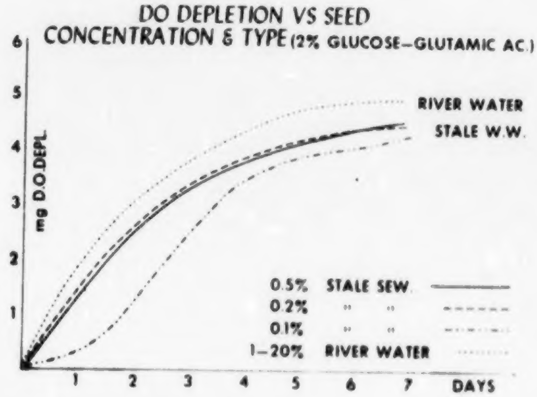


Figure 5

5 It must be recognized that BOD progressions are most likely to err on the low side. A meaningful BOD test should seek the highest consistent oxygen demand feasible for sample and conditions.

D Algae

When large numbers of algae are present in surface waters, they produce significant changes in the oxygen content. Under the influence of sunlight excess oxygen is produced while a net deficit occurs in the dark. The result is a wide variation in surface water DO depending on sample time.

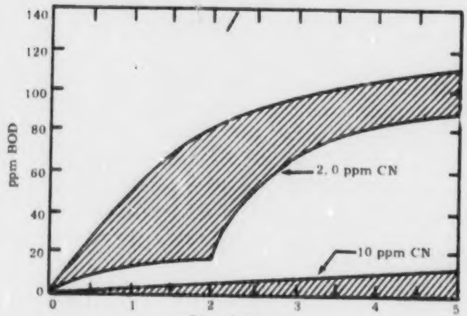
When stream samples containing algae are incubated in the laboratory the algae survive for a time, then die because of the lack of light. Short-term BOD determinations may show the influence of oxygen production by the algae. When the algae die, they release the stored organic load for recycle and increase the BOD. Therefore, samples incubated in the dark may not be representative of the deoxygenation process in the stream, since the benefits of photosynthesis are lacking. Conversely, samples incubated in the light, under conditions of continual photosynthesis, will yield low BOD values.

The influence of algae on BOD is one of the most difficult variables to evaluate. More research is needed to develop satisfactory methods for the accurate determination of BOD in the presence of large numbers of algae. Light and dark bottle incubations suggest the magnitude of effects.

IV TOXICITY

A Effect

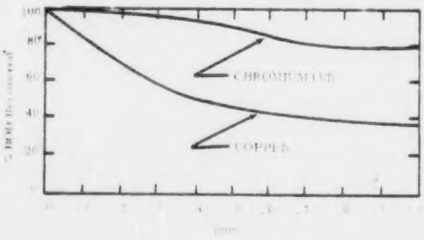
Since satisfaction of the BOD is accomplished through the action of microorganisms, the presence of toxic substances will result in depression of the oxidation rate. In many cases, toxicity will produce a lag period, until tolerant organism activity becomes significant. Figure 6 shows the effect of cyanide on the BOD curve. A prominent lag period is exhibited in the 2 ppm curve, while at 10 ppm the lag extends beyond the fifth day. An activated sludge may be adapted to work effectively in degradation of 60 mg CN/l.



Effect of Cyanide on BOD of Domestic Sewage
(2% Sewage in Formula C Dilution Water)

Figure 6

Heavy metals have similar effects depending on history and environment. The effects of copper and chromium are illustrated in Figure 7.



EFFECT OF HEAVY METALS ON BOD

Figure 7

B Detection

In laboratory determinations of BOD the absence of toxic substances including chlorine must be established before the results can be accepted as valid. Comparison of BOD values for several dilutions of the waste will indicate the presence or absence of toxicity. In Table 1 the calculated BOD for the dilutions show higher values in the more dilute concentrations. It is apparent that toxicity was present and that the toxic effect was diluted out at a waste concentration of 2% or less.

Table I

Waste conc.	Depletion	5 day BOD
10%	3.51	35
5%	4.53	91
2%	2.80	140
1%	1.52	152
0.5%	0.74	148

VII NITRIFICATION

A Mechanism

The oxidation process, as exemplified by the equation:

$$y = L\,(1-10^{-kt})$$

presumably involves the oxidation of carbonaceous matter or 1st stage oxygen demand.

$$\text{C H O} \xrightarrow{O_2} CO_2 + H_2O$$

The rate coefficient is normally high, giving nearly complete oxidation in a few days. When nitrogenous material is present its oxidation can be shown as:

$$NH_3 \xrightarrow{O_2} NO_2^- \xrightarrow{O_2} NO_3^-$$

Nitrogen oxidation may be delayed for several days during BOD tests unless suitable micro-biota are initially available. Under some circumstances these two oxidations can proceed simultaneously and the resultant BOD curve will be a composite of the two reactions.

$$y_t = \left[L_c(1-10^{-k_c t}) + L_n\,(1-10^{-k_n t}) \right]$$

where y_t = the simultaneous BOD of the carbonaceous and nitrogenous phases or 1st and 2nd stage demands.

k_c and k_n = the rate coefficients applicable to the carbonaceous and nitrogenous materials respectively.

L_c and L_n = the ultimate oxygen demands characteristic of the two phases respectively. This is the general formula for any system characterized by two simultaneous reactions.

Principal conditions governing simultaneous carbon and nitrogen oxidation:

1 Presence of an effective nitrifying culture at the beginning of the test interval (nitrifiers grow relatively slowly).

2 Maintenance of adequate DO, believed to be a minimum of 0.5 to 1.0 mg/l, for nitrifier activity.

3 Available nitrogen - in excess of that required for synthesis. This is believed to require a minimum of about 7 mg/l to support active nitrification on a continuous basis.

4 Nitrifiers appear to be more sensitive to toxicity than most saprophytic organisms, hence are likely to be inhibited more readily. This is particularly evident during nitrite to nitrate conversion.

B It may require 5 to 10 days to establish nitrification if the population was not nitrifying initially. This is the basis for the sequential carbonaceous and nitrogenous oxidation of sewage oxidation.

1 Effects on the BOD curve indicate a typical pattern such as in Figure 8. The influence of nitrification in the production of a secondary rise in the BOD curve is so well known that any secondary rise may be erroneously attributed to nitrification whether or not nitrification was involved. Actually, a secondary rise in the curve may be due to any oxidation system assuming dominance after the initial oxidation system has been completed.

2 The nitrification phenomena occurs simultaneously in many streams, treated effluents or partially stabilized samples. The designation of a secondary BOD rise to nitrification should be based on analysis, not curve shape.

C The extent of nitrification is conclusively shown only by periodic analysis of ammonia, organic, nitrite and nitrate nitrogen. The conversion of ammonia and organic nitrogen to oxidized nitrogen is a definite indication of nitrification.

D Nitrification Inhibition

Plant efficiencies from a BOD standpoint can be erroneous because nitrification generally is not established during the usual incubation of influent samples but may be a major factor in effluent incubations. It requires about 2 times the oxygen to convert NH_3 -N to NO_3 -N as to convert C to CO_2 hence this is a major fraction of stream oxygen use. Most secondary treated effluents are characterized by a larger fraction of carbon than nitrogen removal which accentuates the problem.

Pasteurization of samples, methylene blue, chromium, and acid treatment followed by neutralization have been used to inhibit nitrification for estimation of carbonaceous BOD only. Any inhibition of nitrification also produces a change in the sample or its behavior and may partially inhibit carbonaceous oxidation. Nitrification is a factor in stream self-purification and treatment. It does not appear realistic to alter it for convenience. The most realistic approach to carbonaceous oxidation is the measurement of CO_2 or COD.

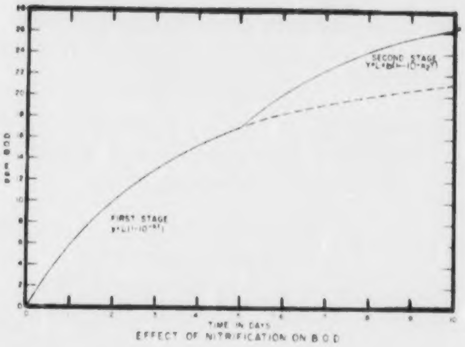


Figure 8

VIII EFFECT OF DILUTION

When a series of dilutions are made on a BOD sample usually the results vary to the extent that only an approximate BOD value is obtained.

Table 2

INTERPRETATION OF BOD DATA

Sample conc.	DO	Depletion	BOD
Initial	8.2	-	-
Final:			
1%	5.5	2.7	270
2%	3.3	4.9	245
4%	0.0	Complete	-

A For example, in Table 2, 1%, 2% and 4% concentrations of sample were used. The 4% concentration became anaerobic before the end of 5 days. The 5-day BOD of the 1% concentration was 270 and that of the 2% concentration was 245.

B Statistically one value is more reliable than the other.

Dilution	DO depletion
1%	5.5 mg/l
2%	3.3 mg/l
Difference	2.2 mg/l

The difference in depletion between 1 and 2% dilutions is 2.2 mg/l. This difference may be attributed to an additional 1% of sample added to the original 1%. If the difference is multiplied by the dilution factor of 100 to obtain the BOD, the result is 220 mg/l.

1 We now have three estimates of the BOD on a one percent concentration basis from the two dilutions:

a the actual 1% depletion gives 270
b 2%/2 depletion gives 245
c (2%- 1%) depletion gives 220

Statistically the probabilities of being nearer the actual value goes with the nearest two of three. The 4% value of 8.2 depletion/4 as a minimum possible BOD 1% concentration gives a BOD of at least 200.

2 There is the possibility that higher concentrations may reflect significant toxicity while lower concentrations tend to reflect a greater proportion of dilution water. The toxicity problem does not appear to be significant since the 4% sample concentration indicated a BOD of at least 200. The higher BOD at 1% sample concentration may be due to a contaminated dilution water or to the fact that a similar number of seed organisms had less food and utilized certain fractions that they had passed by when they had more choice with the 2% sample concentration. Data is insufficient to resolve this one.

3 Incubations having a depletion of at least 2 mg DO/liter and a residual of at least 1 mg DO/liter are indicated to be most valid(1). Both the 1 and 2% concentrations fit this requirement in Table 2. An average error of + or -0.1 ml on the DO titration would have a smaller relative error upon the 2% depletion.

4 We have a reasonable presumption that the sample BOD of about 230 was a good estimate. We do not have an unequivocal basis for so stating. Possible variations in results with different dilutions of a given sample are subject to many uncertainties in the test routine.

If some cause is known - such as a titration eror, the inclusion of extraneous substances producing high or low response, or a definite procedural error that rules out a valid estimate of the sample BOD- that result should be labeled as a lost cause or unreported. Otherwise, report what was obtained to the best of your ability with the provision of uncertainty for uncontrollables.

ACKNOWLEDGMENT:

Certain portions of this outline contain training material from prior outlines by D. G. Ballinger and J. W. Mandia.

REFERENCE:

Standard Methods, APHA-AWWA-WPCF, 13th edition, 1971.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, EPA, WPO, Cincinnati, OH 45268.

Descriptors: Algae, Ammonia, Bacteria, Biochemical Oxygen Demand, Essential Nutrients, Microorganisms, Nitrates, Nitrification, Nitrites, Nutrients, Oxygen Demand, Rates, Time, Toxicity, Waste Dilution

DISSOLVED OXYGEN

Factors Affecting DO Concentration in Water

I The Dissolved Oxygen determination is a very important water quality criteria for many reasons:

A Oxygen is an essential nutrient for all living organisms. Dissolved oxygen is essential for survival of aerobic organisms and permits facultative organisms to metabolize more effectively. Many desirable varieties of macro or micro organisms cannot survive at dissolved oxygen concentrations below certain minimum values. These values vary with the type of organisms, stage in their life history, activity, and other factors.

B Dissolved oxygen levels may be used as an indicator of pollution by oxygen demanding wastes. Low DO concentrations are likely to be associated with low quality waters.

C The presence of dissolved oxygen prevents or minimizes the onset of putrefactive decomposition and the production of objectionable amounts of malodorous sulfides, mercaptans, amines, etc.

D Dissolved oxygen is essential for terminal stabilization wastewaters. High DO concentrations are normally associated with good quality water.

E Dissolved oxygen changes with respect to time, depth or section of a water mass are useful to indicate the degree of stability or mixing characteristics of that situation.

F The BOD or other respirometric test methods for water quality are commonly based upon the difference between an initial and final DO determination for a given sample time interval and condition. These measurements are useful to indicate:

1 The rate of biochemical activity in terms of oxygen demand for a given sample and conditions.

2 The degree of acceptability (a bioassay technique) for biochemical stabilization of a given microbiota in response to food, inhibitory agents or test conditions.

3 The degree of instability of a water mass on the basis of test sample DO changes over an extended interval of time.

4 Permissible load variations in surface water or treatment units in terms of DO depletion versus time, concentration, or ratio of food to organism mass, solids, or volume ratios.

5 Oxygenation requirements necessary to meet the oxygen demand in treatment units or surface water situations.

G The DO test is the only chemical test included in all Water Quality Criteria, Federal, State, Regional or local.

II FACTORS AFFECTING THE DO CONCENTRATION IN WATER

A Physical Factors:

1 DO solubility in water for an air/water system is limited to about 9 mg DO/liter of water at 20°C. This amounts to about 0.0009% as compared to 21% by weight of oxygen in air.

2 Transfer of oxygen from air to water is limited by the interface area, the oxygen deficit, partial pressure, the conditions at the

interface area, mixing phenomena and other items.

Certain factors tend to confuse reoxygenation mechanisms of water aeration:

a The transfer of oxygen in air to dissolved molecular oxygen in water has two principal variables:

  1) Area of the air-water interface.

  2) Dispersion of the oxygen-saturated water at the interface into the body liquid.

  The first depends upon the surface area of the air bubbles in the water or water drops in the air; the second depends upon the mixing energy in the liquid. If diffusors are placed in a line along the wall, dead spots may develop in the core. Different diffusor placement or mixing energy may improve oxygen transfer to the liquid two or threefold.

b Other variables in oxygen transfer include:

  3) Oxygen deficit in the liquid.

  4) Oxygen content of the gas phase.

  5) Time.

  If the first four variables are favorable, the process of water oxygenation is rapid until the liquid approaches saturation. Much more energy and time are required to increase oxygen saturation from about 95 to 100% than to increase oxygen saturation from 0 to about 95%. For example: An oxygen-depleted sample often will pick up significant DO during DO testing; changes are unlikely with a sample containing equilibrium amounts of DO.

c The limited solubility of oxygen in water compared to the oxygen content of air does not require the interchange of a large mass of oxygen per unit volume of water to change DO saturation. DO increases from zero to 50% saturation are common in passage over a weir.

d Aeration of dirty water is practiced for cleanup. Aeration of clean water results in washing the air and transferring fine particulates and gaseous contaminants to the liquid.

e One liter of air at room temperature contains about 230 mg of oxygen. A 5 gal carboy of water with 2 liters of gas space above the liquid has ample oxygen supply for equilibration of DO after storage for 2 or 3 days or shaking for 30 sec.

f Aeration tends toward evaporative cooling. Oxygen content becomes higher than saturation values at the test temperature, thus contributing to high blanks.

3 Oxygen solubility varies with the temperature of the water. Solubility at 10° C is about two times that at 30° C. Temperature often contributes to DO variations much greater than anticipated by

solubility. A cold water often has much more DO than the solubility limits at laboratory temperature. Standing during warmup commonly results in a loss of DO due to oxygen diffusion from the supersaturated sample. Samples warmer than laboratory temperature may decrease in volume due to the contraction of liquid as temperature is lowered. The full bottle at higher temperature will be partially full after shrinkage with air entrance around the stopper to replace the void. Oxygen in the air may be transferred to raise the sample DO. For example, a volumetric flask filled to the 1000 ml mark at 30° C will show a water level about 1/2 inch below the mark when the water temperature is reduced to 20° C. BOD dilutions should be adjusted to 20° C + or - 1 1/2° before filling and testing.

4 Water density varies with temperature with maximum water density at 4° C. Colder or warmer waters tend to promote stratification of water that interferes with distribution of DO because the higher density waters tend to seek the lower levels.

5 Oxygen diffusion in a water mass is relatively slow, hence vertical and lateral mixing are essential to maintain relatively uniform oxygen concentrations in a water mass.

6 Increasing salt concentration decreases oxygen solubility slightly but has a larger effect upon density stratification in a water mass.

7 The partial pressure of the oxygen in the gas above the water interface controls the oxygen solubility limits in the water. For example, the equilibrium concentration of oxygen in water is about 9 mg DO/l under one atmospheric pressure of air, about 42 mg DO/liter in contact with pure oxygen and 0 mg DO/liter in contact with pure nitrogen (@ 20° C).

B Biological or Bio-Chemical Factors

1 Aquatic life requires oxygen for respiration to meet energy requirements for growth, reproduction, and motion. The net effect is to deplete oxygen resources in the water at a rate controlled by the type, activity, and mass of living materials present, the availability of food and favorability of conditions.

2 Algae, autotrophic bacteria, plants or other organisms capable of photosynthesis may use light energy to synthesize cell materials from mineralized nutrients with oxygen released in process.

a Photosynthesis occurs only under the influence of adequate light intensity.

b Respiration of alga is continuous.

c The dominant effect in terms of oxygen assets or liabilities of alga depends upon algal activity, numbers and light intensity. Gross algal productivity contributes to significant diurnal DO variations.

3 High rate deoxygenation commonly accompanies assimilation of readily available nutrients and conversion into cell mass or storage products. Deoxygenation due to cell mass respiration commonly occurs at some lower rate dependent upon the nature of the organisms present, the stage of decomposition and the degree of predation, lysis, mixing and regrowth. Relatively high

deoxygenation rates commonly are associated with significant growth or regrowth of organisms.

4 Micro-organisms tend to flocculate or agglomerate to form settleable masses particularly at limiting nutrient levels (after available nutrients have been assimilated or the number of organisms are large in proportion to available food).

a Resulting benthic deposits continue to respire as bed loads.

b Oxygen availability is limited because the deposit is physically removed from the source of surface oxygenation and algal activity usually is more favorable near the surface. Stratification is likely to limit oxygen transfer to the bed load vicinity.

c The bed load commonly is oxygen deficient and decomposes by anaerobic action.

d Anaerobic action commonly is characterized by a dominant hydrolytic or solubilizing action with relatively low rate growth of organisms.

e The net effect is to produce low molecular weight products from cell mass with a correspondingly large fraction of feedback of nutrients to the overlaying waters. These lysis products have the effect of a high rate or immediate oxygen demand upon mixture with oxygen containing waters.

f Turbulence favoring mixing of surface waters and benthic sediments commonly are associated with extremely rapid depletion of DO. Recurrent resuspension of thin benthic deposits may contribute to highly erratic DO patterns.

g Long term deposition areas commonly act like point sources of new pollution as a result of the feedback of nutrients from the deposit. Rate of reaction may be low for old materials but a low percentage of a large mass of unstable material may produce excessive oxygen demands.

C Tremendous DO variations are likely in a polluted water in reference to depth, cross section or time of day. More stabilized waters tend to show decreased DO variations although it is likely that natural deposits such as leaf mold will produce differences related to depth in stratified deep waters.

ACKNOWLEDGMENTS

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REFERENCE

1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, OH, July 1971.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268 and revised by Charles R. Feldmann, Chemist, National Training Center.

Descriptors: Aeration, Aerobic Conditions, Air-Water Interfaces, Anaerobic Conditions, Benthos, Biological Oxygen Demand, Dissolved Oxygen, Water Pollution, Water Quality

OXYGEN DYNAMICS IN STREAMS

I INTRODUCTION

A Dissolved oxygen is so economically, biologically, and technologically important that its significance is nearly universally recognized. All water quality standards employ dissolved oxygen (DO) concentration as a parameter of water quality.

B Effective water quality management is largely dependent upon control of the natural oxygen balance. Unless pollution control decisions are based upon sound technical knowledge, achievement of desired water quality will be purely fortuitous.

C Full comprehension of the natural oxygen balance is required to:

1 Predict the response of the oxygen resource to any stimulus (including pollution) before it occurs,

2 Assess the effectiveness of existing pollution control measures, and

3 Determine the degree of treatment required to maintain specified oxygen levels.

II EVALUATION METHODS

To efficiently control the oxygen balance one must: (1) account for all processes which influence DO resources, and (2) quantitatively estimate their effects.

A Two methods meet these requirements:

1 Oxygen budget method

This method requires measurement of all oxygen resources and liabilities under a given set of conditions. Should the same conditions reoccur at some future time the observed oxygen values would again prevail.

The budget method has been severely criticized because it tends to be laborious, evaluates the balance only under one set of environmental conditions, and presupposes that methods for independent measurement of the various assets and liabilities exist. Nevertheless, this method may be practical in uncomplicated situations.

2 Mathematical model

The mechanism of oxygen exchange in natural waters could theoretically be described by a mathematical equation or model. A perfect mathematical model would account for all the myriad biological, chemical, and physical factors influencing the oxygen balance and could be used to predict oxygen levels under various conditions.

The mathematical model might be envisioned as a single equation containing numerous terms, each representing the influence of a chemical, physical, or biological variable. In practice, portions of this hypothetical equation have already been developed, but the elusive master equation itself is yet to be derived. At their present state of development, such mathematical models are not fully comprehensive but may be used provided the limitations of the analysis be known.

The values of coefficients and terms of the model may be estimated in the laboratory or derived from field observations.

B The "proof of the pudding" of a budget or model is its ability to accurately reproduce what actually occurs in nature. If the predicted value of DO based on laboratory results or field measurements equals the observed value the budget or model is usually considered to be an accurate reproduction of the natural process. This practice ignores real or intentional compensating errors which do not affect the predicted value but incorrectly indicate the relative effects of individual processes. To be completely accurate a model must not only predict the oxygen sag curve but also accurately compare processes.

C Generally it is easier to estimate the average oxygen exchange for a stream reach than to precisely define the oxygen sag curve. If the former approach may be taken technical constraints may be eased and difficulties much alleviated.

III COMPONENT PROCESSES

Natural oxygen exchange is a complex phenomenon involving several processes. The potential effects of these processes upon the biochemical oxygen demand (BOD) and/or dissolved oxygen (DO) resources of a hypothetical reach of stream are indicated in Table 1. BOD and DO resources are assumed to exist solely within the liquid phase.

Although it would be rare to find less than two processes significant, several of the processes of Table 1 may be inoperative throughout a given reach of stream. One of the major difficulties of oxygen balance analysis is independent estimation of the effects of the individual processes. If acceptable, there are techniques available to estimate the combined effect of several processes.

IV DEOXYGENATION

The following processes increase oxygen liabilities. Effects are normally exerted as oxygen depletion or increased BOD.

A Bacterial Respiration

Bacterial respiration has long been recognized as an oxygen-demanding process and is generally responsible for the majority of natural demand. Although most literature references ascribe this

Table 1. EFFECTS OF SELECTED PROCESSES ON BOD AND DO RESOURCES

BOD		DO		
Addition	Removal	Addition	Removal	BOD and DO, Redistribution
Scour	Sedimentation	Photosynthesis	Immediate demand	
Diffusion from benthal layer	Adsorption	Atmospheric reaeration	Diffusion to the benthal layer	
Local runoff	Volatilization	Local runoff	Purging action of gases	vertical, horizontal, & longitudinal dispersion
Photosynthesis	Respiration of bacteria and zoomicrobes		Plant respiration	
			Respiration of bacteria and zoomicrobes	

demand to "bacteria" it may also include requirements of zoomicrobes of higher tropic levels, such as protozoa and rotifers. Bacterial and zoomicrobe respiration is primarily dependent upon three variables:

1 Nature of compound

a Some organic material, such as domestic sewage, is quite easily degraded by bacteria, whereas other compounds, e.g., lignins and polysaccharides, are relatively resistant. In the presence of a given organism or association of organisms, an organic compound will exhibit a characteristic BOD rate coefficient under standard conditions.

b As the BOD reaction proceeds, the original compounds are converted to materials of lower energy content and availability, shifts occur in microscopic population predominance, and the apparent BOD rate coefficient progressively changes. The BOD reaction may be mathematically described as a combination of several logarithmically linear reactions acting concurrently and/or in sequence.[1, 2]

Accurate prediction of DO levels at all points within a stream reach may require frequent evaluation of the apparent rate coefficient.

c There has been much controversy as to whether nitrification can occur in the presence of organic matter. In the original work of Therriault[3] nitrification followed carbonaceous oxidation. Recent studies by Courchaine[4] and by Downing et al[5] indicate that nitrification may also occur in the presence of organic matter. Carbonaceous matter per se appears to have no intrinsic antagonistic effect upon nitrification but may foster low DO levels, increased cell synthesis, or high levels of predation. As long as DO concentrations exceed 1 mg/l and NH_3 concentration exceeds that required for cell synthesis ammonia appears to function like an individual organic compound during the BOD reaction. Oxygen demand for nitrification may be included in apparently linear BOD reactions.

2 Seed

Some wastes contain bacteria capable of degrading that particular waste. Other wastes are essentially sterile (due to toxic substances, low DO levels, temperature, etc.). In streams receiving waste discharges over a long period of time naturally occurring bacteria are usually present which are capable of degrading the waste material.

Many compounds have been classified as "non-degradable" in a biological environment. The variety of organisms existing in nature, their adaptive powers, and the processes controlling effective exposure time are so complex that, in the absence of contrary evidence, it is perhaps conservatively advisable to assume that any organic compound may be degraded within a stream reach. Many "non-degradable" compounds are biologically utilized at such low rates that conventional analytical techniques do not detect reaction progress within practical observation periods. Also, many of the processes which affect oxidation in nature cannot be accurately duplicated in the laboratory.

Bacterial respiration is normally evaluated by BOD bottle tests or Warburg or large-volume respirometer studies.

B Immediate Demand

Immediate oxygen demand is exerted spontaneously upon contact of waste components with oxygen. The demand reaction is normally complete within 15 minutes and is exerted solely in the liquid phase.

Immediate demand is especially harmful because its effects are exerted at the instant or location of discharge, rather than being distributed over a reach of stream as would be the case with BOD. Fortunately, this type of demand is not common.

C Benthal Demand

A large portion of stream oxygen demand may be derived, not from bacterial-zoomicrobe respiration in the soluble phase, but from anaerobic-aerobic reactions in organic bottom sludges. Such deposits affect the oxygen balance in two ways.

1 They exert an oxygen demand through aerobic respiration within the benthal layer. Oxygen for this process must be supplied by diffusion from the liquid phase.

2 They release to the overlying liquid simple organic compounds which exert additional BOD.

Although the rate of aerobic oxidation in the sludge mass is generally low the total amount of unoxidized material in the deposit may be so large that the oxygen resources of the soluble phase become severely taxed. The significance of organic sludge deposits is dependent upon the aerobic-anaerobic respiration rates and the amount of oxidizable material in the sludge mass.

Benthal demands are determined by measurement of oxygen depletions in dark bell jars[6] or special benthal respirometers,[7] or by deduction from observed field data.

D Purging Action of Gases

Extremely active benthal deposits may liberate gases, such as H_2S, which cause immediate oxygen demand. This demand is difficult to evaluate separately, and is usually included in determinations of benthal demand.

E Plant Respiration

Oxygen requirements for plant respiration may exceed all other demands if growths are significant. Unfortunately, this is commonly the case. Whether plant respiration rates are affected by oxygen concentration remains a controversial question. Assumption of constant respiration rates has proven acceptable in numerous field studies but controlled laboratory research increasingly indicates that respiration rates are affected.

Respiratory requirements are measured in dark BOD bottles,[8] dark large-volume respirometers, dark algal chambers,[9-10] or by field observation during darkness.[11]

F Scour

During periods of high flow or turbulence organic matter which had previously deposited on the stream bottom may be re-suspended and exert BOD at an increased rate. The combination of potentially large sludge deposits and an increased rate of exertion may cause exceptionally severe oxygen depletion.

Scour is usually evaluated by comparison of the observed BOD, COD, or organic carbon profile with the predicted profile based on BOD bottle or respirometers results.

G Land Runoff

Surface runoff may contribute considerable BOD to a stream. For modeling purposes it is assumed that the load is contributed in a uniform manner along the stream reach.

Normally this load is only a short-term liability during periods of rainfall.

V REOXYGENATION

The following processes enhance oxygen assets. Effects are normally exhibited as increased DO or decreased BOD.

A Natural Reaeration

This source is usually the most important factor in supplying dissolved oxygen to receiving waters. Some of the factors affecting reaeration:

1 Oxygen deficit

When oxygen is removed from solution, an imbalance is created and the deficiency is made up by atmospheric oxygen passing into the solution. The rate of reaeration is proportional to the dissolved oxygen deficit and may be expressed as follows:

$$\frac{dD}{dt} = -K_2D \qquad (1)$$

where: K_2 = Reaeration coefficient (base e)

D = Oxygen deficit = oxygen at saturation - actual oxygen content (mg/l)

2 Physical and chemical factors of the receiving stream

a Roughness of the stream bed - Roughness of a stream bed imparts turbulence to the stream flow and enhances all gas transfer operations (including reaeration).

b Width or surface area - Allows for more surface contact between water surface and atmosphere.

c Depth - In general deep streams have lower reaeration coefficients than do shallow streams.

d Velocity - Swift moving water will tend to be more turbulent and will enhance more efficient reaeration.

e Solubility - Various wastes may affect the solubility of oxygen in the receiving water. This aspect may readily be determined in the laboratory. An example of this is the effect chloride concentration has on oxygen solubility in water.

f Pollution - Oily wastes may form a blanket or surface film on the water surface and reduce transfer of oxygen from air to the water.

3 O'Connor and Dobbins[12] have postulated formulas for approximation of the reaeration coefficient of natural rivers and streams. Two cases were considered:

a Non-isotropic turbulence where a vertical velocity gradient and shear stress exist (characteristic of shallow streams).

b Isotropic turbulence where neither a significant velocity gradient nor shearing stress exist (characteristic of deep streams).

The appropriate formulas for calculation of K_2 (at 20° C) under nonisotropic and isotropic conditions, respectively, are

$$(K_2)_{20^\circ C} = \frac{48.6\ (s)^{1/4}}{H^{5/4}} \qquad (2)$$

and

$$(K_2)_{20^\circ C} = \frac{13.0\ V^{1/2}}{H^{3/2}} \qquad (3)$$

where: K_2 = reaeration coefficient (base e) under stated conditions

V = velocity of stream flow (ft/sec)

H = stream depth (ft)

s = slope of stream bed (ft/ft)

These formulas are based upon a molecular diffusion coefficient, D_L, for oxygen in water of 0.00195.

c Determination of the type of flow existing in the river or stream is made on the basis of the magnitude of the Chezy, or roughness, coefficient of the channel, defined as follows:

$$C = \frac{V}{\sqrt{Hs}} \qquad (4)$$

If the value of C is less than 17 the flow is considered to be non-isotropic, and if C is greater than 17, isotropic.

d The K_2 derived for a temperature of 20°C may be corrected to other temperatures as follows:

$$(K_2)_{T°C} = (K_2)_{20°C} \times 1.0241^{(T-20)} \qquad (5)$$

where T = temperature, °C

4 O'Connor and Dobbins' isotropic formula has recently been substantiated by Tsivoglou et al.[13] Based on measurements of air-water transfer of radioactive argon, an experimentally confirmed ratio between the gas transfer rates of argon and oxygen, and determination of travel time with fluorescent tracers, these workers have estimated K_2 independently of all other natural processes.

B Photosynthesis

Oxygen derived from photosynthesis may constitute a large fraction of the DO resource. Whether this production should be considered an asset is problematical. Since photosynthesis is not operative during darkness, supply of excess DO during the daytime may be of little advantage. On the other hand, it may be beneficial if nighttime depletions are considered insignificant.

1 If the oxygen budget method is used, photosynthetic production is usually measured as gross photosynthesis, P. The respiratory demand of plant metabolism is usually included in measurements of community respiration.

2 If a mathematical model is used, photosynthetic production is assumed to equal the difference between plant respiration and photosynthesis, (P-R). This value is usually derived from analysis of field data.

Gross photosynthesis may be measured by the light-dark bottle method.[9] Net photosynthesis, (P-R), must be derived from field data.[11, 14] Plant respiration has not been measured independently.[15]

C Sedimentation

Sedimentation removes oxidizable matter from solution and reduces the BOD of the liquid phase. This effect is usually evaluated by comparison of the field BOD profile with the predicted profile based on laboratory determinations.[16, 17]

D Biological Extraction

Growths on the stream bottom may remove BOD from solution by adsorption. This effect is evaluated in the same manner as sedimentation.[17] If sedimentation and biological extraction occur in the same reach, it may be difficult to determine their effects independently.

VI MATHEMATICAL EXPRESSION

The cumulative effects of deoxygenation and reoxygenation produce a characteristic variation in streams called the "dissolved oxygen sag curve." This curve has been described by several equations.

A Streeter-Phelps

1 In 1925 Streeter and Phelps[18] proposed the following equation:

$$D = \frac{K_1 L_A}{K_2 - K_1}\left(e^{-K_1 t} - e^{-K_2 t}\right) + D_A e^{-K_2 t} \qquad (6)$$

where

D = dissolved oxygen deficit (mg/l)

t = time of flow (days)

L_A = ultimate carbonaceous BOD at the upstream end of the reach (mg/l)

D_A = dissolved oxygen deficit at the upstream end of the reach (mg/l)

K_1 = BOD rate coefficient determined in the laboratory (per day)

K_2 = reaeration coefficient (per day)

2 This equation assumes that

- a The stream flow is steady and uniform.
- b The process for the stretch as a whole is a steady-state process. The conditions at every cross section being unchanged with time.
- c The BOD removal process may be described by a single rate coefficient throughout the entire stream reach.
- d The removal of oxygen by the benthal demand and by plant respiration, the addition of oxygen by photosynthesis, and the addition of BOD from the benthal layer or the local runoff are all uniform along the stretch.
- e The BOD and oxygen are uniformly distributed over each cross section, thus permitting the equations to be written in the usual one-dimensional form.
- f The ultimate carbonaceous BOD represents all oxygen liabilities which will be exerted within the stream reach.

Since these conditions are unlikely for great lengths the stream is usually modeled in successive, short reaches.

3 BOD removal coefficients may be determined by bottle tests, respirometer experiments, or derivation from the actual BOD profile.

- a If BOD removal is solely a function of bacterial respiration, the rate coefficient derived from bottle or respirometer experiments may be acceptable.
- b If other factors are known to affect the removal process the rate should be derived from the field profile. Derivation from the field profile constitutes a preferred procedure.
- c Either bottle tests, respirometer experiments, or the field profile may reveal a rate coefficient progression which may be approximated by a sequence of linear reactions.[1] If such a progression exists, prediction of the actual profile requires application of different coefficients and ultimate BOD values to the several sub-reaches.

4 Values of ultimate BOD for substitution into the equation may be estimated by three methods:

- a Extension of a semi-logarithmic plot of short term BOD bottle results. Combinations of 3-day and 7-day[9] or 2-day and 5-day[19] results have been employed.
- b Addition of the oxygen equivalent of the organic and ammonia nitrogen to COD results.[20]

c Addition of the oxygen equivalents of ammonia and organic nitrogen and organic carbon.

5 If nitrification is known to occur within the reach (or sub-reach), its potential demand should be either wholly included in the estimate of L or wholly included in another term.

6 The overall change in BOD within the reach may be estimated by addition of carbonaceous demand based on observed rates and estimates of ultimate first-stage demand to stoichiometric oxygen requirements[15] for observed nitrate production.

7 The Streeter-Phelps equation is quite adequate for profile or overall change prediction in simple environments, and may be utilized to predict overall changes in complex environments. It is generally inadequate for prediction of profiles in complex situations.

B Thomas' Approach

Thomas[16] has proposed that the rate coefficient expressing natural BOD removal be split into two fractions as follows:

$$K_r = K_1 + K_3 \tag{7}$$

where:

K_1 = reaction coefficient determined in the laboratory

K_3 = coefficient expressing the effects of other variables

The corresponding form of the Streeter-Phelps would be

$$D = \frac{K_r L_A}{K_2 - K_r} \left(e^{-K_r t} - e^{-K_2 t} \right) + D_A e^{-K_2 t} \tag{8}$$

When organic matter (BOD) is removed by ancillary factors, K_3 is positive; when it is returned to the flowing water, to be measured at a downstream station, K_3 may be negative.

C Dobbins' equation

1 Dobbins[21] has recently proposed an equation which accounts for several oxygen exchange processes:

$$D = \frac{K_1 \left(L_A - \frac{L_a}{K_r} \right) \left(e^{-K_r t} - e^{-K_2 t} \right)}{K_2 - K_r} + D_A e^{-K_2 t} + \left(\frac{D_B}{K_2} + \frac{K_1 L_a}{K_2 K_r} \right) \left(1 - e^{-K_2 t} \right) \tag{9}$$

where

D = dissolved oxygen deficit (mg/l)

t = time of flow (days)

L_A = ultimate carbonaceous BOD at the upstream end of the reach (mg/l)

D_A = dissolved oxygen deficit at the upstream end of the reach (mg/l)

K_1 = BOD removal rate coefficient as determined by bottle or respirometer tests

K_2 = reaeration rate coefficient (per day)

K_r = overall coefficient of BOD removal derived from the field profile (per day)

L_a = rate of addition of BOD along the reach (mg/l/day)

D_B = the net rate of removal of oxygen by the benthal demand and the effect of plants (mg/l/day)

2 This equation is based on the following assumptions.

a The stream flow is steady and uniform.

b The process for the stretch as a whole is a steady-state process.

c The removals of BOD by both bacterial oxidation and other processes are first order reactions.

d The removal of oxygen by the benthal demand and by plant respiration, the addition of oxygen by photosynthesis, and the addition of BOD from the benthal layer or the local runoff are all uniform along the stretch.

e The BOD and oxygen are uniformly distributed over each cross section, thus permitting the equations to be written in the usual one-dimensional form.

f The ultimate carbonaceous BOD represents all oxygen liabilities which will be exerted within the stream reach.

3 Ultimate BOD values and rate coefficients may be estimated by methods suitable for the Streeter-Phelps equation.

4 Nitrogenous demand may be evaluated by inclusion in the ultimate BOD value or calculation from observed nitrate production.

5 Although this equation represents a significant improvement over other forms, determination of the values of the several constants and terms may prove difficult. The effects of diffusion of oxidizable matter from the benthal layer, for example, may be included in the value of either L_a or D_B. In either case, this effect would be combined with that of other processes. This equation represents the most rational expression of the DO balance yet presented.

VII LONGITUDINAL DISPERSION

A The equations previously presented assume that all incremental volumes of liquid which originated at a given cross section reach a downstream section at the same time, i.e. that the flow time of every incremental volume exactly equals the distance divided by the mean stream velocity. However, an unknown fraction of the incremental volumes may reside in the reach longer than the average flow-through value, be exposed to the BOD reactions longer, and cause greater DO depletion at a downstream point than would be expected during the average travel time, t.

B Dobbins[21] has presented an equation which accounts for longitudinal dispersion:

$$D = \frac{K_1 \left(L_A - \frac{L_a}{K_r} \right) \left(e^{mx} - e^{rx} \right)}{K_2 - K_r} + D_A \; e^{rx} + \left(\frac{D_B}{K_2} + \frac{K_1 \; L_a}{K_2 \; K_r} \right) \left(1 - e^{rx} \right) \qquad (10)$$

where

D, t, L_A, D_A = terms previously described

K_1, K_2, K_r, L_a (Section VI C1)

D_B

$$m = \frac{U - \sqrt{U^2 + 4 \, K_r \, D_L}}{2 \, D_L} \qquad (11)$$

$$r = \frac{U - \sqrt{U^2 + 4 \, K_2 \, D_L}}{2 \, D_L} \qquad (12)$$

U = mean stream velocity (ft/day)

D_L = coefficient of longitudinal dispersion (sq. ft. /day)

C Based on the ratio of mx (which expresses the rate of BOD removal in a dispersion-affected system) to $K_r t$ (which expresses BOD removal in a non-turbulent system), Dobbins has presented rather convincing evidence that effects of longitudinal dispersion will rarely be significant in flowing streams. In slow-moving, well-mixed streams dispersion may be significant.[22]

VIII SUMMARY

Sound analysis of the natural oxygen balance is necessary to accurately predict the oxygen profile in streams or to assess the influence of various factors upon oxygen resources. Two methods of analysis are available: the budget method and mathematical models. Several examples of the latter have been presented. In addition, the processes which affect oxygen exchange have been identified and methods for evaluation of their effects have been indicated.

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This outline was prepared by F.P. Nixon, formerly Acting Regional Training Officer, Northeast Regional Training Center, Hudson-Delaware Basins Office, Edison, NJ 08817.

Descriptors: Biochemical Oxygen Demand, Dissolved Oxygen, Equations, Mathematical Models, Oxygen Demand, Oxygen Sag, Photosynthetic Oxygen, Reaeration, Water Quality Control

EFFECT OF BENTHIC DEPOSITS ON DISSOLVED OXYGEN RESOURCES

I INTRODUCTION

A Objectionable sludge deposits are now commonly prohibited by regulatory authorities. The final report of the National Technical Task Committees on Water Quality Criteria[1] recommends that sludge deposits be prohibited in receiving waters rated for all major water uses.

B Such extensive control is largely based on widespread recognition of the potential oxygen demand of benthal deposits. Although large benthal demands have frequently been observed, prediction of their magnitude and incidence is difficult. The magnitude and incidence of benthal loads depend upon the amount and nature of putrescible matter in the waste and the frequency and duration of periods of deposition.

C During periods of high flow or turbulence biodegradable sludges may become resuspended and deplete overlying waters of oxygen. Thus a body of oxygen deficient water may pass downstream and harm fish and other aquatic life for a considerable distance. Many feel that this effect is more dangerous than exertion of a continuous, albeit unsuspected demand.[3]

II EFFECTS

A Benthal deposits affect oxygen resources in two ways:

1 They exert an oxygen demand through aerobic respiration within the benthal layer. Oxygen for this process must be supplied by diffusion from or mixing with liquid phase.

2 They release to the overlying liquid simple organic compounds which exert additional BOD. The composite rate of biological degradation in the overlying liquid may increase or decrease downstream of the area of deposition.

B Anomalies in the dissolved oxygen (DO) sag curve may be caused by deposition. The apparent effect, depicted in Figure 1, is similar to that of an unsuspected outfall.

III DEPOSITION

A The potential for deposition may be evaluated by examination of a plot of cumulative time of passage versus distance along the stream reach, as shown in Figure 2. Deposition may be expected in those sections where velocity is less than a critical value. Velz[2] recommends a critical velocity for sedimentation of 0.6 ft/sec and a critical velocity for sour of 1.0-1.5 ft/sec. The magnitudes of these velocities would depend upon the nature of the waste and may need to be determined empirically.

B Deposition potential is usually evaluated at critical flow.

IV MAGNITUDE OF DEMAND

Although the location of potential benthal demand is rather easily determined,

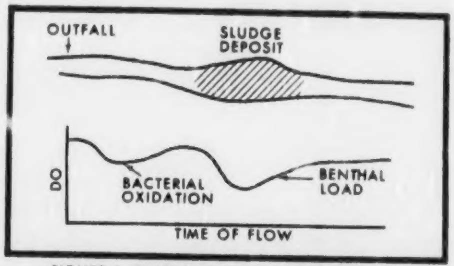

FIGURE 1. TYPICAL DO SAG CURVE BELOW AN OUTFALL DISCHARGING SETTLEABLE ORGANIC WASTE

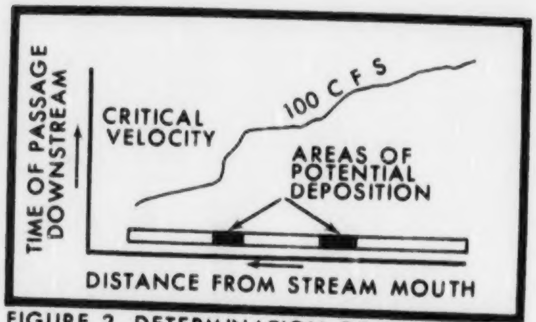
TIME OF PASSAGE DOWNSTREAM
CRITICAL VELOCITY
100 C F S
AREAS OF POTENTIAL DEPOSITION
DISTANCE FROM STREAM MOUTH

FIGURE 2. DETERMINATION OF DEPOSITION POTENTIAL

evaluation of its magnitude is more difficult. Benthal demand is dependent upon the nature of the waste and the amount of accumulation in the area of deposition. Velz[2] has proposed a procedure by which the effects of the colloidal and dissolved portion of a waste may be evaluated separately from those of the settleable portion.

A The ultimate biochemical oxygen demand (BOD) of the settleable fraction is assumed to be the difference between the ultimate BOD of the raw waste and the ultimate BOD of a sample allowed to settle for one hour.

B The aerobic removals of BOD of the colloidal and dissolved and the settleable portions are plotted separately, as shown in Figure 3. Rates of aerobic satisfaction are either assumed or determined experimentally. Rate coefficient progressions may occur in either phase.

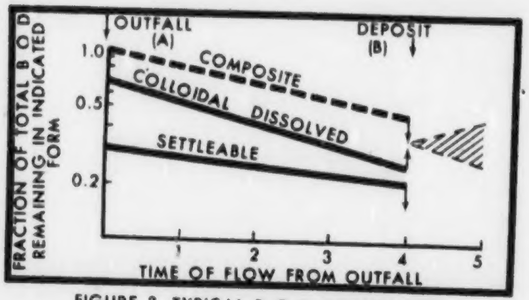

FIGURE 3. TYPICAL B O D INTEGRATION

C At the point of deposition the remaining settleable matter becomes wholly incorporated in the benthal mass. If the retention time of the sludge mass is large, the demand exerted may nearly equal the ultimate BOD of the daily addition of settleable matter.

1 The removal of BOD from the deposit will equal the amount satisfied by aerobic oxidation plus the amount released to the overlying liquid. Carbonaceous matter released to the overlying liquid as methane (CH_4) may escape to the atmosphere or be utilized in further bacterial metabolism.

2 The factors which influence the relative importance of these processes are extremely complex and not well understood. Management decisions are commonly based on the assumption that the oxygen demand exerted on the overlying waters equals the ultimate BOD of the daily addition to the deposit.

3 Nitrification becomes more established as the age of the deposit increases. Moore indicates that a large portion of the total organic nitrogen content of "young" deposits is liberated as ammonia whereas NH_3 evolution from "old" deposits is minimal.

D Downstream of the benthal deposit the composite BOD rate coefficient may increase or decrease depending upon the amount of elution, the nature of the compounds released, and the presence of acclimated organisms. Several environmental variables, especially temperature and stream flow, influence this response.

V SUMMARY

Benthal deposits affect oxygen resources by exerting an aerobic demand within the benthal layer or by releasing to the overlying water compounds which exert additional BOD. The location of areas of potential deposition may be determined by examination of time of passage curves based on critical flow. Aerobic benthal demand is assumed to equal the ultimate BOD of the daily addition to the sludge mass.

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Descriptors: Benthos, Biochemical Oxygen Demand, Oxygen Demand, Sedimentation, Sewage Sludge, Sludge

EFFECTS OF AQUATIC PLANTS ON DISSOLVED OXYGEN RESOURCES

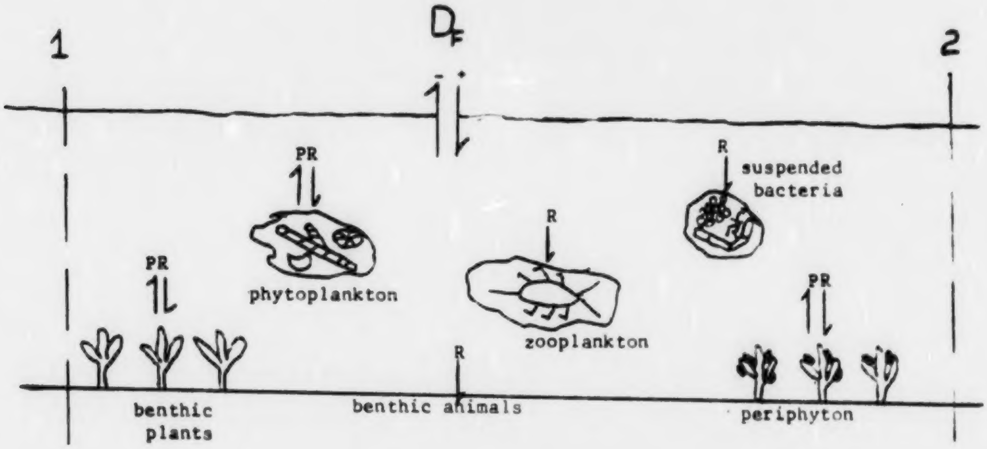
I INTRODUCTION

A Various workers have emphasized the effects of aquatic plants on oxygen resources. Camp[1] reported that two-thirds of the dissolved oxygen (DO) resource of the Merrimack River is derived from photosynthesis; Hull[2, 3] reported similar results for Baltimore Harbor and the Delaware River estuary.

B Other investigators have found aquatic plants detrimental to the dissolved oxygen resource. Gunnerson and Bailey[4] and O'Connell and Thomas[5] have reported extreme depletions and rapid DO fluctuations in waters containing large benthos and phytoplankton populations.

II BASIC PROCESS

A The oxygen concentration of a body of water is the composite effect of addition and depletion of oxygen from solution by biological and physical processes, shown in Figure 1.



(1) $$O_2 = (\Delta O_2)_{BIOL} \pm D_F$$

(2) $$(\Delta O_2)_{BIOL} = P_{\substack{\text{benthic}\\\text{plants}}} - R_{\substack{\text{benthic}\\\text{plants}}} + P_{\text{phytoplankton}} - R_{\text{phytoplankton}} - R_{\substack{\text{suspended}\\\text{bacteria}}} - R_{\text{zooplankton}} + P_{\text{periphyton}} - R_{\text{periphyton}} - R_{\substack{\text{benthic}\\\text{animals}}}$$

FIGURE 1. OXYGEN DYNAMICS IN RECEIVING WATERS

1 Equation (1) indicates that the change in value of the oxygen resource equals the net effect of all biological processes, $(\Delta 0_2)_{BIOL}$, plus or minus gas transfer at the water surface, D_F.

2 Equation (2) indicates that the net effect of all biological processes equals the sum of oxygen production by photosynthesis, $P_{x, y, \ldots}$ and oxygen depletion by respiration, $R_{x, y, \ldots}$ for the several habitats or classes of organism present, X, Y, . . . Some organisms have no photosynthetic capability and exert only a depleting effect.

3 Terms of equations (1) and (2) may be expressed as time rates of change (g/sq m/hr, mg/l/hr) or as increments of amount or concentration (lb, mg/l).

B In the presence of significant plant growth the several photosynthetic and respiratory processes combine to produce the classical dissolved oxygen variation shown in Figure 2. The classical curve reaches a maximum during mid-afternoon and a minimum just before sunrise. DO concentration may exceed the 24-hr. mean for a large portion of the day. During mid-afternoon DO may exceed saturation concentration and oxygen will be vented to the atmosphere.

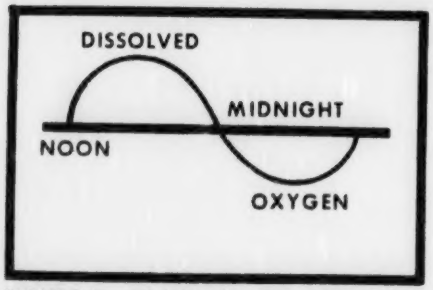


FIGURE 2. CLASSICAL DO VARIATION

III ESTIMATION

A Engineers are commonly asked to estimate two quantities:

1 The fraction of the total DO resource which is attributable to aquatic plants, and

2 The contribution of aquatic plants to community respiration.

3 In a complex environment it is very difficult to independently estimate the effects of each class of organism. Effects are usually estimated collectively for identifiable groups, such as plankton, benthos, or the entire community. Several methods have been developed to estimate the plant contributions; none exists for independent measurement of plant respiration.

4 If a single organism is predominant within a reach the measured overall effect is usually attributed to that class. The degree of predominance must be independently demonstrated.

B Photosynthetic Production

The amount of oxygen added to a body of water by aquatic plants equals the gross production of oxygen by photosynthesis less the oxygen requirement for plant respiration and the amount of oxygen vented to the atmosphere. (P-R) is often called net production for the habitat or class of organism involved.

1 Values of P, R, and (P-R) may be determined for planktonic communities by light-dark bottle techniques.[1, 2, 3]

a Gross photosynthesis, P, equals the DO change in a bottle incubated under light minus the change in a bottle incubated in darkness.

b Planktonic respiration, R, equals the DO depletion in a bottle incubated in darkness. If both bacteria and suspended algae are present the depletion will be the result of the activities of both types of organisms.

c Net production equals the DO change in a bottle exposed to light and darkness for the same periods observed in the natural environment.

d Estimation of net production by light-dark bottle techniques may require submergence of several sets of bottles in the stream itself. In this manner natural conditions are closely duplicated.

e The light-dark bottle technique is more representative if DO concentration does not exceed saturation. The technique may be affected by nutrient supply, lack of turbulence, or oxygen concentration.

2 Odum's[6] method

Net production may also be determined by correction of the observed DO variation for atmospheric diffusion.

a The diurnal variation of oxygen is plotted for a point or a stream reach as shown in Figure 3A.

b For each incremental sampling period the rate of change of oxygen concentration is calculated and plotted as in Figure 3B.

c Based on an assumed or determined exchange coefficient the rate of atmospheric 0_2 transfer is plotted as in Figure 3C.

d Summation of curves 3B and 3C will produce a curve of the rate of net production, Figure 3D. This curve will indicate when respiration exceeds production. If the average value exceeds zero, oxygen is added to the flowing water by aquatic plants.

e This technique includes the effects of all influences and groups of organisms. The effect of the benthic community may be estimated by subtraction of light-dark bottle results from the overall production values estimated by Odum's method.

C Plant Respiration

Techniques for independent estimation of plant respiration have not yet been developed. Composite respiration values may be estimated by dark bottle or dark algal chamber techniques or by field measurement of oxygen exchange during darkness.

IV SUMMARY

The effect of aquatic plants on oxygen resources is widely recognized. Such plants have been called both beneficial and detrimental. This classification is usually based upon the rated use of the stream and the intensity of the diurnal oxygen variation. Techniques are available for estimation of net production, (P-R), for plankton, benthos, or the entire community. Respiration can be determined only collectively for several groups. The basic principles of two major estimating techniques have been presented.

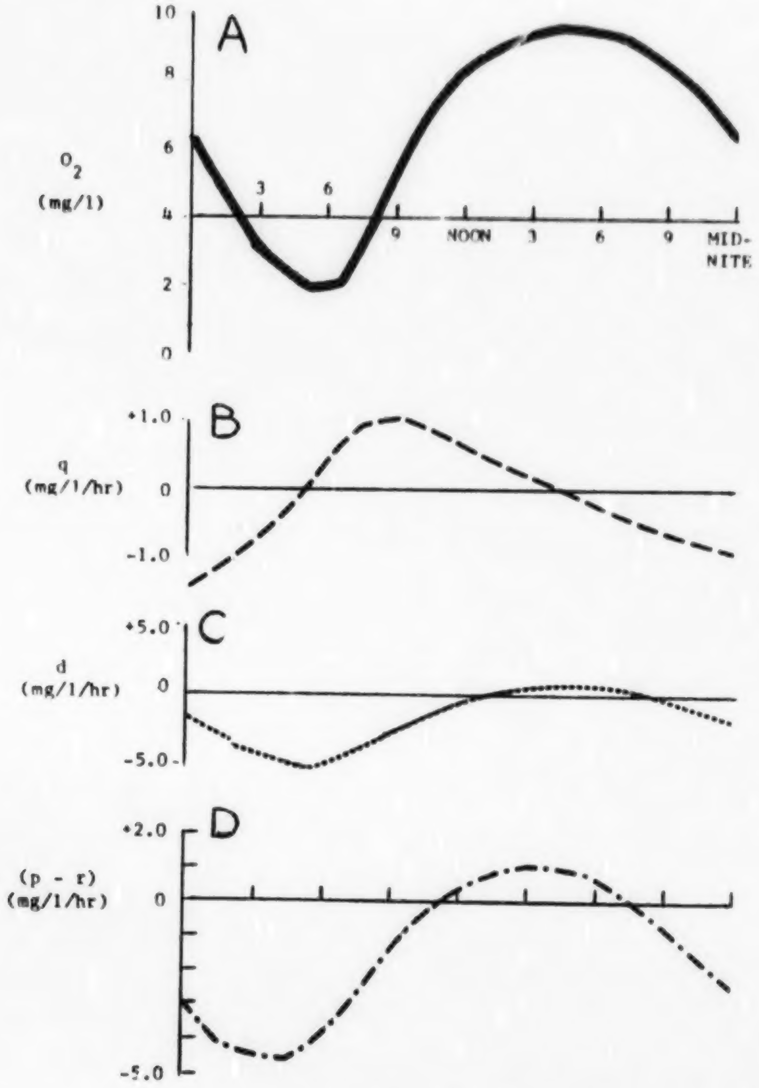


FIGURE 3. DERIVATION OF (P - R)

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Descriptors: Aquatic Plants, Aquatic Populations, Aquatic Productivity, Dissolved Oxygen, Dirunal Distribution, Oxygen Demand, Photosynthetic Oxygen, Productivity, Respriation

ORGANIC ENRICHMENT AND DISSOLVED OXYGEN RELATIONSHIPS IN WATER

I INTRODUCTION

A Oxygen normally composes approximately 20% of the atmosphere, but from only 0.5 to 1% of unpolluted water by volume (9 - 14 milligrams per liter, or parts per million).

B Physically, oxygen is a colorless gas, responsive to the laws of solubility, temperature, etc.

C The amount of oxygen present can be readily determined by various methods, both chemical and physical.

D Biologically, oxygen is one of the key elements to all life, all living organisms contain it along with hydrogen and carbon. It is the physiological "complement" of carbon dioxide.

E When properly interpreted, oxygen concentration can serve as an excellent index to water quality.

1 It is widely present.

2 It is intimately involved in many processes, chemical, physical, and biological.

II BIOLOGICAL OR PHYSIOLOGICAL RELATIONSHIPS (Figure 1)

A Photosynthesis is the biological starting point for synthesizing living substance out of non-living carbon dioxide, water, and other materials. In the process, radiant energy is adsorbed from the sun (hence it is an endothermic reaction) and free molecular oxygen is released to the environment as long as sufficient light is available, i.e., during daylight only.

1 The conventional empirical formula for photosynthesis is a very great simplification, as many as twenty steps may actually be involved (like respiration, controlled by systems of enzymes).

$$6\ CO_2 + 6\ H_2O + 673\ \text{Cal}$$

(Energy sunlight)

$$\Big\downarrow\ \text{chlorophyll (enzyme system)}$$

$$C_6H_{12}O_6 + 6\ O_2$$

(Simple sugar)

2 The end product is a basic organic material known as a simple sugar (carbohydrate, monosaccharide, of which "glucose" is an example, see Figures 1, A) which serves as a starting point for the elaboration of higher organic substances, and which may also be oxidized or "respired" by the cell to release the stored chemical energy.

3 Synthesis of organic materials (living protoplasm) from simple sugars and other substances in bacteria, algae, and man.

a Glucose and other simple sugar molecules can be combined by living organisms such as algae into other carbohydrates such as starch.

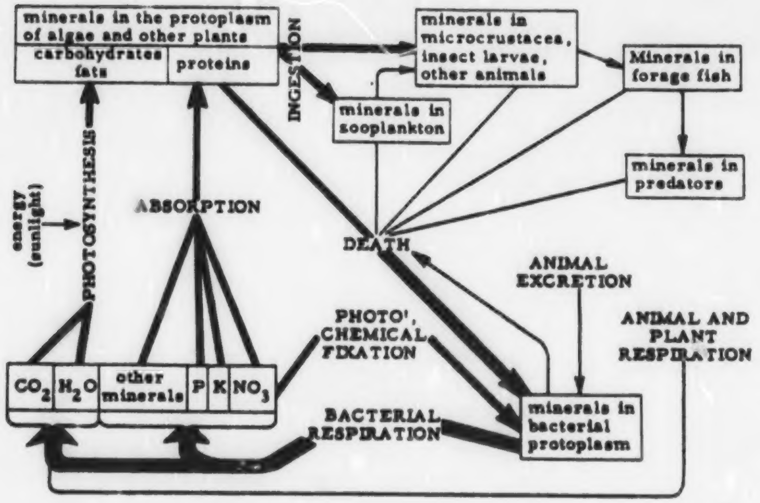
b After modification into fatty acids and glycerol, fats can be made.

c Combination with nitrogen and other minerals taken in from the surrounding water produces amino acids. These can then be combined into proteins, the most complicated of all life substances. Plants are the only organisms that can synthesize amino acids completely "from scratch."

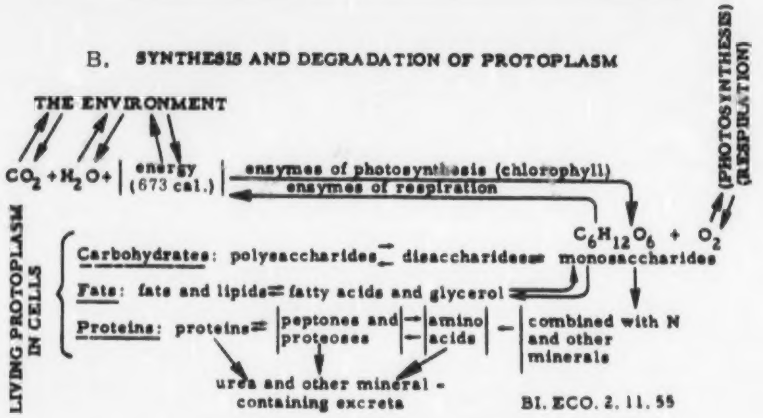
ENERGY CYCLES

H. W. Jackson

A. THE GENERAL FOOD CYCLE

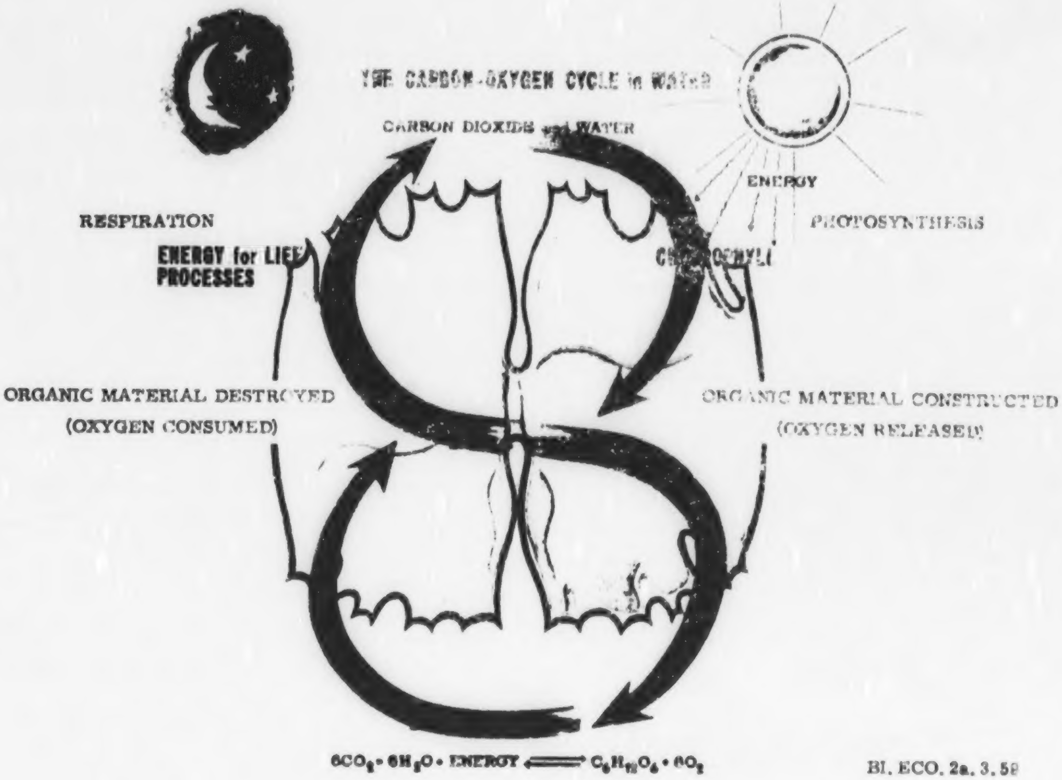

minerals in the protoplasm of algae and other plants
carbohydrates fats
proteins
INGESTION
minerals in microcrustacea, insect larvae, other animals
Minerals in forage fish
minerals in zooplankton
minerals in predators
PHOTOSYNTHESIS
energy (sunlight)
ABSORPTION
DEATH
ANIMAL EXCRETION
ANIMAL AND PLANT RESPIRATION
PHOTO', CHEMICAL FIXATION
CO_2
H_2O
other minerals
P
K
NO_3
BACTERIAL RESPIRATION
minerals in bacterial protoplasm

B. SYNTHESIS AND DEGRADATION OF PROTOPLASM


THE ENVIRONMENT
$CO_2 + H_2O +$
energy (673 cal.)
enzymes of photosynthesis (chlorophyll)
enzymes of respiration
$C_6H_{12}O_6 + O_2$
(PHOTOSYNTHESIS)
(RESPIRATION)
LIVING PROTOPLASM IN CELLS
Carbohydrates: polysaccharides ⇌ disaccharides ⇌ monosaccharides
Fats: fats and lipids ⇌ fatty acids and glycerol
Proteins: proteins ⇌ peptones and proteoses ⇌ amino acids ← combined with N and other minerals
urea and other mineral - containing excreta

BI. ECO. 2. 11. 55

Figure 1


THE CARBON-OXYGEN CYCLE in WATER
CARBON DIOXIDE and WATER
ENERGY
RESPIRATION
PHOTOSYNTHESIS
ENERGY for LIFE PROCESSES
CHLOROPHYLL
ORGANIC MATERIAL DESTROYED (OXYGEN CONSUMED)
ORGANIC MATERIAL CONSTRUCTED (OXYGEN RELEASED)
$6CO_2 + 6H_2O + ENERGY \rightleftharpoons C_6H_{12}O_6 + 6O_2$

BI. ECO. 2a. 3.59

Figure 2

4 All of these synthetic or growth processes are endothermic and require energy to consummate. This energy is obtained by respiration.

B Respiration. Life processes such as those mentioned previously (II A 3, also physical movement) are energized by the controlled oxidation or "respiration" of single sugars. This is a cellular or physiological process and continues constantly in all living things, independently of light, as long as life persists.

1 Types of respiration. Respiration is an energy releasing or exothermic biochemical process and is to be distinguished from the external bodily act of "breathing", which is a process involving a lung, gill, or other organ, whereby molecular oxygen is brought inside the body of the organism and carbon dioxide is released. Breathing is transportation; respiration is burning or biochemical utilization.

a Aerobic respiration. The most common form of respiration is illustrated by the oxidation of glucose, using free dissolved oxygen from the environment. This is also the most efficient type since virtually all of the chemical energy contained in the glucose molecule is released (673 Cal.) and the residue is completely mineralized or stabilized to carbon dioxide and water.

Although actually taking place in more than twenty steps involving as many different enzymes, the overall process can be empirically represented by the following formula:

$C_6H_{12}O_6 + 6\,O_2$

(a simple sugar such as glucose)

$\downarrow$

$6\,CO_2 + 6\,H_2O + 673$ Cal

(enzymes) (energy)

b Anaerobic respiration. If free dissolved oxygen (DO) is not available from the environment, certain types of bacteria, fungi, and other organisms known as "facultative aerobes" can switch to another, less efficient type of respiration and still obtain energy by breaking down simple sugars. This is known as anaerobic respiration. Some types of microorganisms, the true anaerobes, can thrive only under anaerobic conditions.

The anaerobic respiration of glucose involves splitting the molecule without the use of outside oxygen so as to release some of the contained energy, but not all. The remainder is still available in the end products. A common empirical relationship is as follows:

$3\,C_3H_{12}O_6 \longrightarrow 2\,CH_3(COOH)_2$

(glucose) (enzymes) (lactic acid)

$+\,2\,CH_3CH_2OH\ +\,2\,CH_3\,COOH$

(ethyl alcohol) (acetic acid)

$+\ \dfrac{59\ 1/2\ \text{Cal}}{(\text{energy})}$

c Anaerobic respiration of proteins and their derivations by microorganisms (known as putrefaction) often leads to the production of foul smelling end products. Aerobic respiration of proteins (known as decay) seldom produces offensive odors. In addition to carbohydrates, proteins, fats, and various other natural and unnatural hydrocarbons, and other substances can also be broken down or respired to relatively stable end products by microorganisms.

2 The adaptability of life is remarkable. By the same mechanisms of genetics and natural selection which have resulted in the evolution of present day life on earth, microorganisms have evolved which are capable of obtaining energy from, or in other words, "respiring" a great variety of new synthetic materials, the products of modern industry. This is the basis for the biological treatment of wastes.

a The types of biological mechanisms involved, in general, are known and to a degree, understood.

b Many species or kinds of organisms may be involved in the stabilization of a single type of waste.

3 Significance of respiration in sanitary engineering. The process of respiration uses or "demands" oxygen from the environment. Any material, such as sewage, an organic industrial waste, motor oil, etc., which will support life of any kind will thus exert (through the organisms living on it) a "BOD".

a Respiration has two end "products": energy, by which the organisms live; and degraded, mineralized, or stabilized material such as digested sewage sludge which retains little or no energy available to organisms. That is, they have no biochemical oxygen demand or BOD remaining.

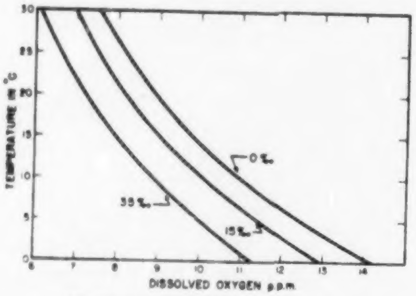
b Since it is independent of light, it goes on night and day.

III PHYSICAL PHENOMENA

A Solubility of oxygen in pure water varies with temperature and pressure according to well known laws and values.

1 We expect to find less oxygen in warmer waters and more in colder. This has both seasonal and geographical implications.

2 Dissolved substances such as salts tend to reduce the solubility of oxygen at a given temperature (See Figure 3 - Oxygen Solubility at Selected Salinities).



OXYGEN SOLUBILITY AT SELECTED SALINITIES

Figure 3

3 The concentration of oxygen often is expressed as percent of saturation.

a Organisms, however, are affected by the real quantities present, and actually demand the most oxygen at the highest temperature, when the solubility is lowest.

b It is therefore more significant, where organisms and stream conditions are concerned, to express concentration as milligrams per liter (or parts per million).

B The movement or distribution of oxygen throughout a water mass is not entirely dependent on molecular diffusion, but also involves various types of gross water movements. Reaeration (or aeration) is the transfer of oxygen from the air into the water mass.

IV CHEMICAL FACTORS

A Chemical toxicity may delay the exertion of biological oxygen demands, or the release of oxygen by biological mechanisms.

B Chemical substances may themselves demand oxygen from the water.

1 If oxygen is available, the chemical oxygen demands tend to be satisfied relatively quickly and hence are likely to be a more local problem.

2 They are independent of any biological effect.

3 They are in addition to biological action.

V OXYGEN AND EUTROPHICATION

A Diurnal interactions of photosynthesis and respiration. (Figure 6)

1 Life, growth, and hence the need for oxygen for respiration continue twenty-four hours a day, as long as life persists.

2 Since photosynthesis in nature is activated by radiant energy from the sun, it is operative only during the daytime. It releases approximately twenty times as much oxygen as is consumed in cellular respiration, however, so there is a great excess left over which diffuses out into the surrounding water. Here it is available to biochemical oxidative demands of other organisms with the result that the ecological system becomes "aerobic."

3 DO's affected by photosynthesis thus tend to be highest in the daytime (particularly mid-afternoon) and lowest at night (3 - 4 A.M.).

B Organic materials from domestic sewage or similar wastes are readily available to microorganisms. Assimilation of food, and growth and multiplication of the population can thus begin relatively quickly, thus establishing a demand for oxygen for respiration.

1 It takes a significant period of time for a population of bacteria, fungi, and other microorganisms such as protozoa to become established, after the initiation of growth of the few original organisms, even under the best of conditions.

2 Dissolved oxygen does not thus disappear instantly from the water upon the admixture of sewage, but rather begins to diminish slowly. As the population of oxygen consuming organisms builds up, the deficit in the water increases. As this occurs, any equilibrium which may have existed with the atmosphere is destroyed, and the rate of aeration through the surface film increases.

a As the population of microorganisms grows still larger, its rate of increase begins to increase (logarithmic growth). Very soon a size of population is achieved where the demand for oxygen exceeds the amount which can be supplied through the surface film. The concentration of free DO now begins to drop sharply and may go to zero.

b If sufficient food for the microorganisms remains, anaerobic conditions will now prevail. Oxygen, of course, continues to enter at a maximum rate through the surface film, but is immediately used up. The overall rate of oxidation of the waste or food material however, is now very low (although the total amount may be large), as surface aeration can supply but a small portion of the oxygen needed.

c If there is no replenishment of the food supply by repollution, the big population of fungi and bacteria eventually uses up most of the available substrate or food, and begins to starve. Growth now begins to slow down, and the population eventually drops back to its original low level.

d Meanwhile time has passed. An abundance of fundamental plant nutrients such as nitrate, phosphate, potash, etc., have been released to the water as a result of microbial respiration. Algae begin to grow on this substrate, and increase rapidly to tremendous numbers.

e Algal photosynthesis thus suddenly begins to release great quantities of oxygen in the daytime. At first this is exhausted each night by respiration of the algae and other microorganisms, but soon persists around the clock until the algae in turn have exhausted their food supply and return to numbers normal for an unpolluted stream.

f With the above mentioned advent of free DO from the algae, the more efficient aerobic type of respiration can again be employed by the microorganisms. This hastens the oxidation or stabilization process, and leaves behind but a minimum residue of well mineralized material to accumulate on the bottom.

g It should be mentioned that turbidity from suspended microorganisms and other organic solids frequently inhibit the establishment of an algal population until the biochemical oxidation or stabilization process has been well started. Turbidity inhibits algal growth primarily by shading and suppression of photosynthesis.

C When organic material is dumped into a body of water faster than it can be oxidized by the mechanism described above, it tends to accumulate on the bottom in a partially digested condition known as sludge.

1 Great quantities of nutrient energy are still present, as well as a huge population of microorganisms. There is a very great need for oxygen which, if available, is avidly taken up by the organisms in the surface layers.

a Aerobic conditions in the overlying waters thus tend to hasten the stabilization of sludge banks by providing oxygen for aerobic type respiration.

b Anaerobic waters, on the other hand, tend to preserve the sludge by restricting all organisms to anaerobic types of respiration. It has been reported that the speed of decomposition begins to be restricted at concentrations below 1 ppm.

2 Anaerobic conditions are present throughout the sludge mass below the surface. Stabilization is still proceeding, but at a much slower rate than if oxygen were present.

D In the deeper reservoirs, lakes, rivers, and estuaries thermal stratification may develop in the summer.

1 The epilimnion or upper layer, being in contact with the atmosphere, receives a continual replenishment of oxygen by aeration processes.

2 If turbidity is not excessive, photosynthesis by algae (Figure 5) will also provide oxygen.

3 The hypolimnion or lower layer, being separated from the atmosphere and frequently from the light, tends to acquire an excess of carbon dioxide and a deficiency of oxygen due to the respiratory activities of microorganisms (such as protozoa, fungi, and bacteria). (Figure 4)
This effect is heightened if the bottom material is high in organic content as has been shown below.

Figure 4
Non-Pigmented, Non-Oxygen-Producing, Protozoan Flagellates

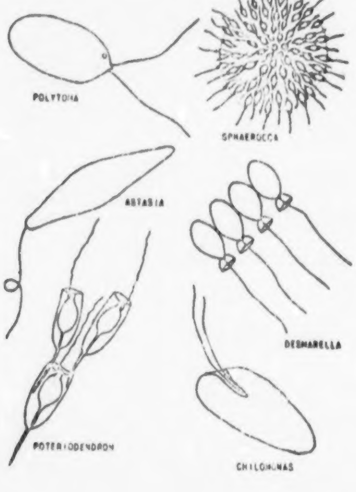
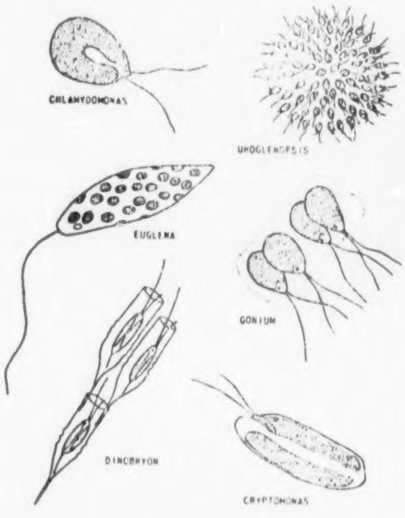


Figure 5
Pigmented, Oxygen-Producing, Algal Flagellates



VI PROBLEMS AND BENEFITS RESULTING FROM ALGAE PRODUCTION (Figures 4, 5, 6)

A Problems to man may result when the total "primary production" by algae leads to an increase in the total organic content of the water that interferes with a desired use.

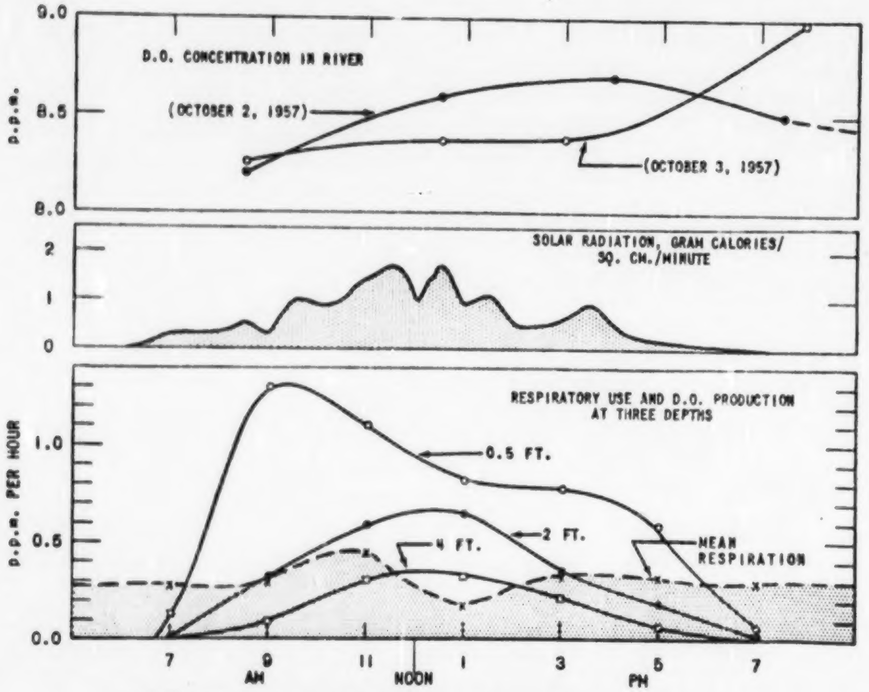
1 This may consist of a high algal population that produces a water with high turbidity, taste and odor, or other undesirable effect. High respiratory needs may lead to nocturnal oxygen deficit.

2 Certain algae may cause tastes and odors, clog filters; or otherwise interfere with potable water processing.

3 Death of large algal populations may lead to obnoxious odors through bacterial decomposition. Oxygen deficits may result at any time of day in this process. Deposition of masses of organic sediment or sludge in estuaries and back waters may be considerable.

B The primary production of algae can also serve as a supply of food to consumer organisms (animals), resulting in increased production at several (trophic) levels: (Figure 7)

zoömicrobes, microinvertebrates, macroinvertebrates, fishes.



DISSOLVED OXYGEN RELATIONS IN OHIO RIVER (REACH 11, BROMLEY)
OCT. 3, 1957

Figure 6

**Mean Surface Water Productivity Rates of the Great Lakes
and Other Freshwater Lakes and Ocean Areas.**

Lake or Ocean and Location	Productivity Rate in mg.C/ m^3 /day	Investigator
Lake Superior	16.62	Parkos (1967-68)
Lake Huron	23.04	Parkos (1968)
Lake Michigan	37.62	Parkos (1967)
Lake Erie	175.20	Parkos (1968)
Grand Traverse Bay, N.E. Lake Michigan	0.34	Saunders et al. (1962)
Douglas Lake, Mich.	0.23	Saunders et al. (1962)
Bras d'Or Lake, Nova Scotia, Canada	23.5	Geen and Hargrave (1966)
Brooks Lake, Alaska	3.2	Goldman (1960)
Maknek Lake, Alaska	8.8	Goldman (1960)
Torne Träsk, Sweden	6.4	Rodhe (1958b)
Ransaren, Sweden	8.8	Rodhe (1958b)
Lake Erken, Sweden	221.0	Rodhe (1958b)
Lake Esrom, Denmark	1600	Jonasson and Mathiesen (1959)
Lake Furesø, Denmark	2100	Jonasson and Mathiesen (1959)
N.E. Atlantic - near Denmark	21	Steemann-Nielsen (1960)
North Atlantic, same lat. as Great Lakes	7.5	Steemann-Nielsen (1958b)
Southeast Pacific	3.3	Holmes (1961)
Southwest Pacific	7.2	Angot (1961)
North Pacific, Sub-Arctic Region	6.4	Koblentz-Mishke (1961)

Figure 7

1 Earlier notation cited the release of oxygen during utilization of CO_2 during algal photosynthesis. This encourages fungal or bacterial breakdown of pollutants.

2 Photosynthesis occurs in the presence of adequate light and favorable conditions. In darkness, the cells continue to respire and may consume more oxygen than they produced because photosynthesis increases the organic load.

3 Photosynthesis tends to occur at the surface where light intensity is greatest (Figure 6). Poor vertical mixing would result in stratification of water supersaturated with oxygen over oxygen deficient water. Depending upon conditions, a significant fraction of the oxygen could be lost to the atmosphere.

ACKNOWLEDGEMENTS:

This outline contains certain material submitted by F.J. Ludzack and M.E. Bender.

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This outline was prepared by H.W. Jackson, Chief Bilogist, National Training Center, Office of Water Programs, EPA, Cincinnati, Ohio 45268

Descriptors: Algae, Biochemical Oxygen Demand, Chemical Oxygen Demand, Dissolved Oxygen, Eutrophication, Microorganisms, Oxygen, Oxygen Demand, Oxygenation, Photosynthesis, Photosythetic Oxygen, Respiration

AERIAL RECONNAISSANCE IN POLLUTION SURVEILLANCE

I INTRODUCTION

A Definition

The word "reconnaissance" is derived from the word "reconnoiter" which means to conduct a preliminary examination or survey. Its earlier applications to engineering and military requirements has been expanded to include photomapping and interpretations of natural resources. Aerial reconnaissance can be defined as "airborne examination or survey procedures performed by heavier-than-air craft, lighter-than-air craft, or earth orbiting satellites."

B Types of Aerial Reconnaissance

1 Visual

Examination of the flight path by a human observer with no provision for permanent recording for later study. This form of aerial observation has limited use but in many cases can complement the other forms.

2 Image forming sensor

Image recording of the covered flight path where maximum advantage can be made of image interpretation techniques. Image forming sensors includes such instruments as cameras, infrared scanners, and radar.

3 Nonimage forming sensors

Nonimage forming sensors include such devices as oscilloscopes, strip charts, and dial indicators which directly indicate parameter differentials as received by the sensing elements from the target scan.

4 Combination image forming and nonimage sensors

It is advantageous at times to combine both of these techniques in order to rapidly interpret flight path imagery. This advantage is shown in Figure 1 where a heated effluent discharge is traced to a canal by infrared imagery and indicated by the "lighter" plume which parallels the shoreline. The oscilloscope phototracing indicates the temperature differential of the discharge point as compared to the offshore bay area.

C Detected images can be identified by one or more of the following criteria.

1 Size of image

2 Shape of image

3 Tonal qualities of image

4 Image profile shadowing

5 Location of image

6 Texture patterns of grouped images

7 Spatial relationship of image to surrounding bodies

D Selection of Remote Sensing Method

1 A knowledge of the electromagnetic spectrum (Figure 2) is important in order to select the most advantageous method of remote sensing. It will be noted that there are transmissibility and sensitivity limits as well as existing film limits with respect to equipment wave length patterns.

2 Remote sensing methods can be categorized into two areas.

a Source active: Utilization of an instrument which is capable of emitting a source of energy which is transmitted to the target area and emanations are received whose characteristics are dependent upon the nature of the specific target.

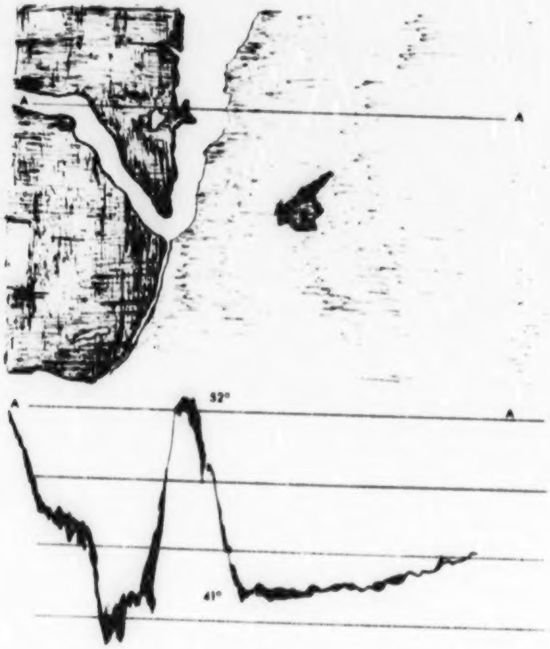

FIGURE 1

An example of such a device is the Radar set (Figure 3) or special Infrared Emitters both of which are capable of being utilized in total darkness.

b Source passive: Utilization of instrumentation which is only capable of receiving target area emanations. Such remote sensing instruments, therefore, do not transmit artificial energy sources but depend upon the sun as a source of energy which is selectively received and individually reflected by each object in the target area. Aerial photography and most of the remote sensing is by this method and the included regions of the electromagnetic spectrum are the upper portions of the UV band to the near infrared region.

3 Selection of desired wavelength is dependent upon the optimum emission capability of the instrument which has the shortest wavelength possible to sharply differentiate small objects while being long enough to preclude excessive energy "scattering" and yet not long enough so that photographic recording is impossible.

Utilization of camera systems (Multiband) exposing discrete bands of the photographic spectrum simultaneously have allowed the photo-interpreter to make discriminations which are not possible with single bands if black-and-white photography. The interpreter may use various projection devices which allow the viewing of one, two, or more images superimposed for a more sophisticated analysis capability.

II TECHNIQUES

A Radar(RAdio Detection And Ranging)

Radar is particularly useful for discerning certain types of vegetation which may not appear in the best visual quality in Panchromatic Color Film (sensitive to the

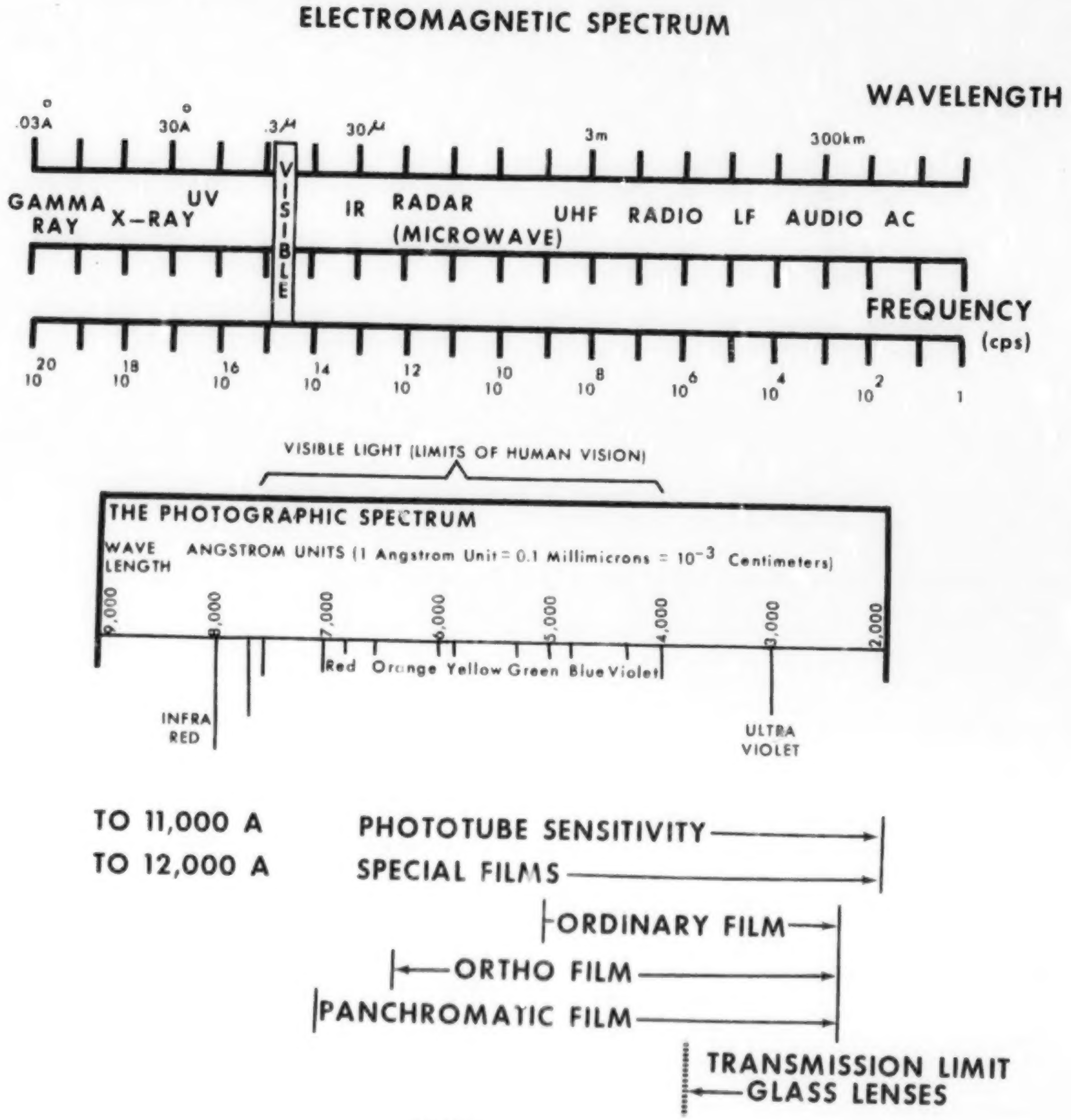
ELECTROMAGNETIC SPECTRUM
WAVELENGTH
.03Å
30Å
.3µ
30µ
3m
300km
GAMMA RAY
X-RAY
UV
VISIBLE
IR
RADAR (MICROWAVE)
UHF
RADIO
LF
AUDIO
AC
FREQUENCY (cps)
10^20
10^18
10^16
10^14
10^12
10^10
10^8
10^6
10^4
10^2
1
VISIBLE LIGHT (LIMITS OF HUMAN VISION)
THE PHOTOGRAPHIC SPECTRUM
WAVE LENGTH
ANGSTROM UNITS (1 Angstrom Unit = 0.1 Millimicrons = 10^-3 Centimeters)
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
Red
Orange
Yellow
Green
Blue
Violet
INFRA RED
ULTRA VIOLET
TO 11,000 A
PHOTOTUBE SENSITIVITY
TO 12,000 A
SPECIAL FILMS
ORDINARY FILM
ORTHO FILM
PANCHROMATIC FILM
TRANSMISSION LIMIT
GLASS LENSES

FIGURE 2

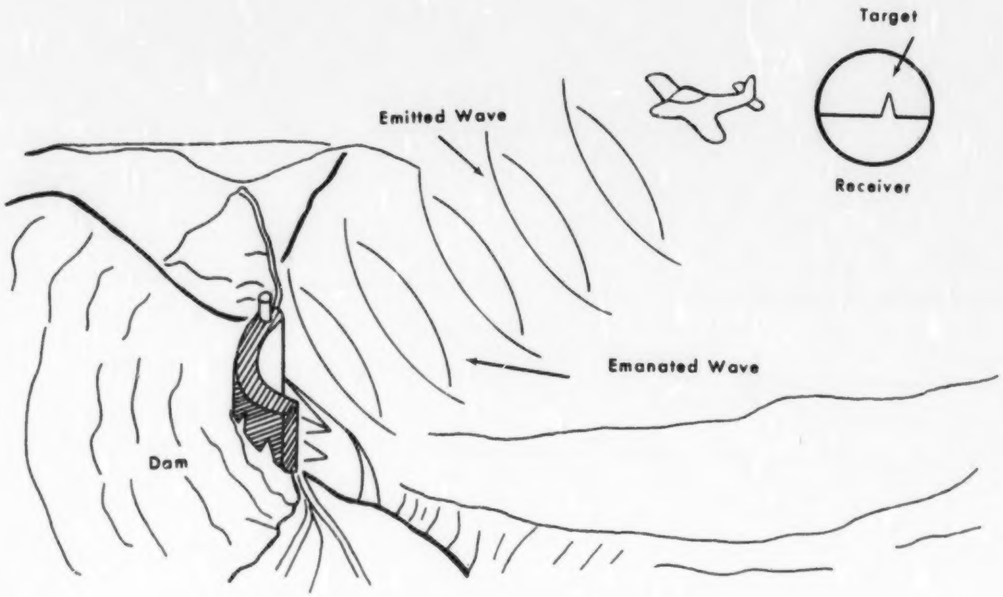

FIGURE 3

entire visible spectrum) and also has all-weather and around-the-clock capabilities. Radar in the longer wavelength ranges is capable of penetrating dense vegetative cover and this advantage has been found to be useful in detecting drainage networks and geological features.

B Conventional Photography

Conventional photography covers the entire range of the visible spectrum and employs both black-and-white film and color film. Some of the special films are capable of photographing a portion of the infrared band (the "near" infrared region) and this capability is useful in special situations.

1 Direct photomapping and panoramic scan

Figure 4 illustrated the manner in which direct photomapping differs from the panoramic scanning technique and it can be seen that continual attached mapping scans can be made of all or portions of the flight path to be analyzed.

2 Multiband photography

Multiband photography is, as its name implies, the synchronized photography of up to nine separate photographs of the same target area each of which is photographed in a different wavelength. A distinct advantage of this method is that, during photo analysis, distinct anomalies may be evident between objects appearing in the different bands and further investigation may be warranted. An example of this is shown in Figure 5 where it was observed that an anomaly in vegetative growth was apparent in the Infrared wavelength while not being apparent in the other visual spectral ranges. Further investigations proved that gravel washings in slug intervals had affected normal growth of the streamside vegetation.

3 Infrared photography

Infrared photography has been found useful in depicting certain hydrological features. Within the photographic

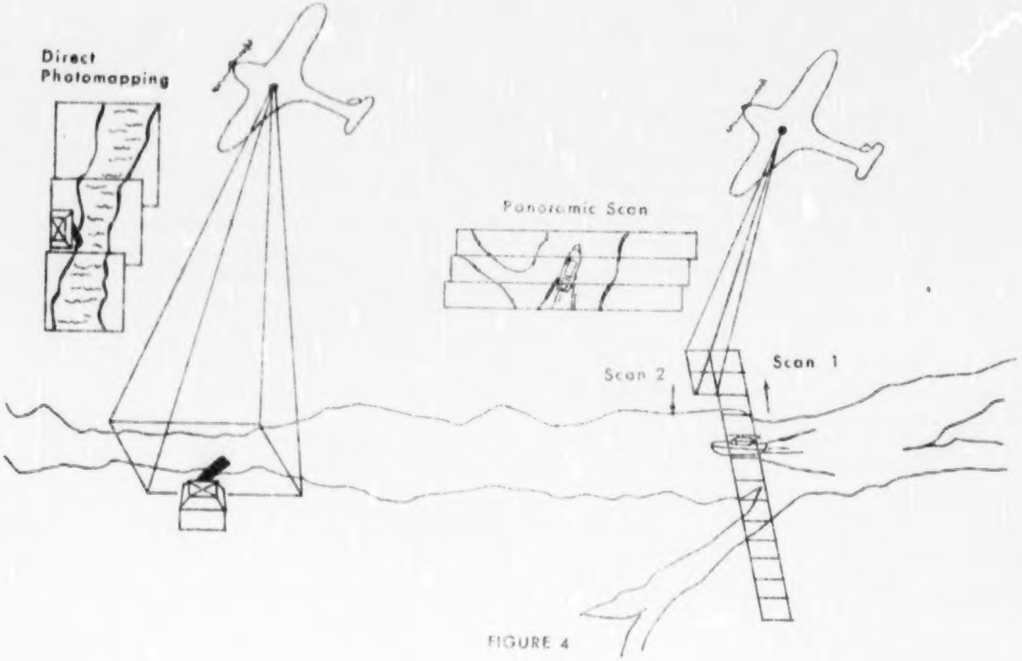
Direct Photomapping
Panoramic Scan
Scan 2
Scan 1

FIGURE 4

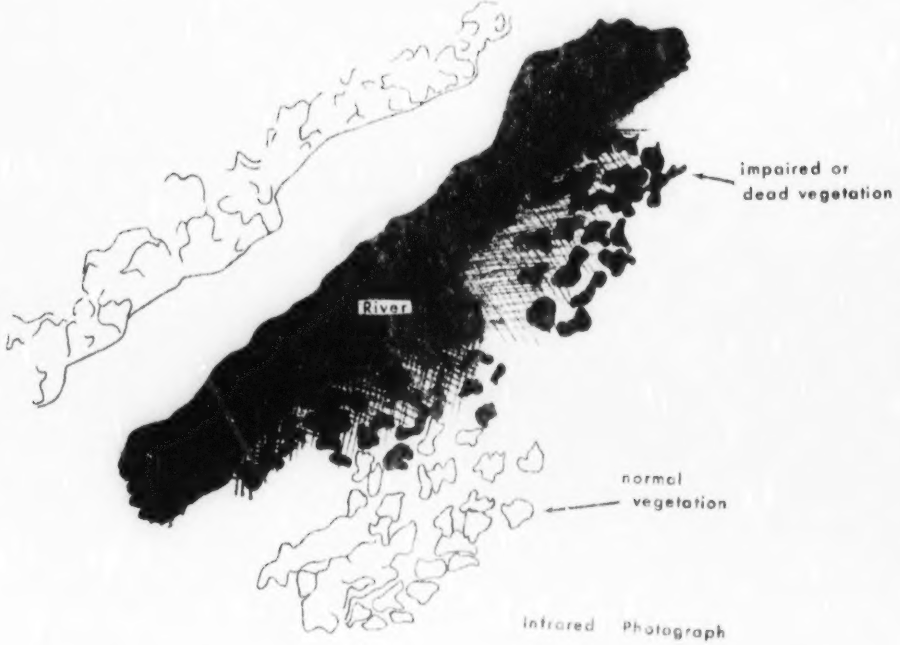
impaired or dead vegetation
River
normal vegetation
Infrared Photograph

FIGURE 5

ranges it is not a heat-measuring tool and therefore cannot detect thermal anomalies within bodies of water but instead sharply delineates shoreline and tributary features and has haze penetrating qualities. The blues appear much darker by infrared photography and the reds, greens, and yellows will show up much lighter when compared to a panchromatic color photograph.

4 Gamma Ray Spectrometer

This device functions in the very short wavelengths and has been found to have excellent detecting capabilities for radioactivity. Such a device would serve as an excellent means of searching for radioactive waste spills which have occurred either accidentally or intentionally.

C Infrared Radiometry

Infrared imagery is different than infrared photography since the tone of the imagery is directly related to the infrared radiation emitted from the target area. This type of scanner converts the normally unphotographic IR band to a thermal imagery. An optical-mechanical device is necessary since the normal thermal energy emitted by the camera itself will influence radiations received from the target area and this elimination of detector internal radiation is accomplished by miniturization and application of extremely low temperatures. One example of the usefulness of infrared imagery is shown in Figure 6 where the dark areas along the shoreline were found to be caused by cold water infiltration by a previously unknown source. Such a finding could be indicative, for example, of a new untapped freshwater source or a polluting influence from a remote source. This cold water infiltration was not evident by normal visual spectrum photography and the IR photograph would only indicate a more distinct shoreline.

D Automatic Analysis

In the analysis of large numbers of prints use can be made of photoelectric scanners wherein automatic recording of degree of brightness can be recorded on a tape printout. An example of this procedure is shown in Figure 7 where each symbol has a significance relating to "tone signature." The "D" zones are the darker areas while the other symbols relate to lighter zones. In the center the clear area is a river tributary. Future predictions are that automatic encoding can be channeled to computers wherein decisions can be made, for instance, in water management policies.

E Stereoscopic Analysis

The principles and methods developed during World War II for the stereoscopic analysis of aerial photographs has been a continuing and expanding science. Its principles are based upon the fact that the human eyes are normally spaced about 65 millimeters apart and are capable of compositing, via the optic nerve, the two separate and distinct images viewed by each orbit. Aerial stereoscopic analysis depends upon the placing of two photographs precise distances apart with precise overlapping as required by normal human visual acuity. With the aid of special stereoscopic viewing lenses the viewer is able, in most cases, to composite both of the photographs in the same manner as with normal vision and thus a sense of depth can be imparted materially enhancing the analyzing of the terrain.

III APPLICATIONS

Aerial reconnaissance in pollution surveillance can be accomplished in a variety of ways and the method chosen usually is dictated by costs involved and what is desired with regard to immediate or potential information.

A Visual Observations

The simplest and least expensive method is to visually observe the study area and this can be useful in determining sampling sites, locating previously undetermined tributaries, pinpointing visual gross

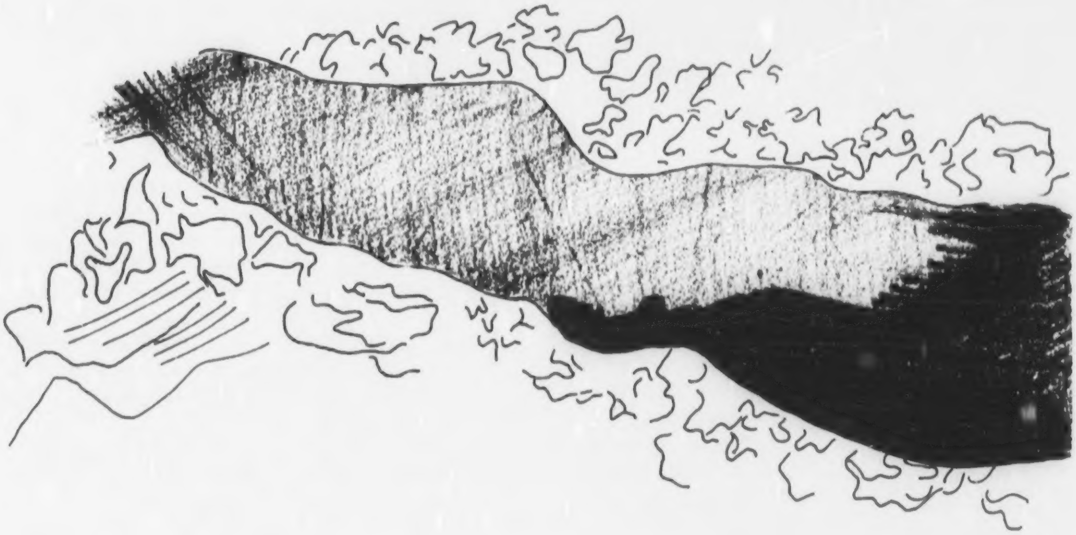

FIGURE 6

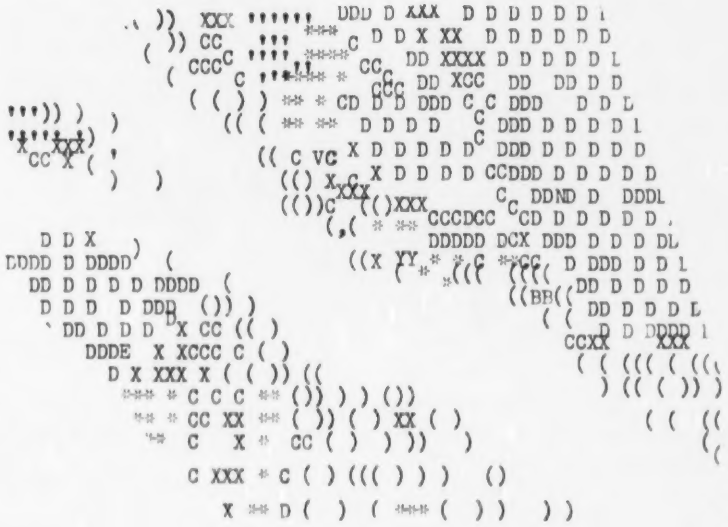

FIGURE 7

pollutant effluents, etc. To augment these observations it is usually better to have a permanent recording of the survey area in the form of photographic or imagery recordings.

B Photographic Techniques

Photographic evidence, either color, black-and-white, or special film, can be recorded in various attitudes or positions of the aircraft in order to make best use of interpretation techniques such as stereoscopic analysis.

1 Current patterns and flow velocity can be ascertained by a method developed by the U.S. Coast and Geodetic Survey whereby powdered aluminum is surface distributed to a wide area and subsequent photographic patterns can be analyzed. Flowing bodies can either appear as "depressions" or "elevations" depending upon direction of flow with relation to the photographing aircraft and from these "parallax" anomalies the velocity can be determined.

2 Photographic IR has found applications in the areas of detecting sharp delineations of shorelines and tributaries especially when haze is prevalent.

3 The work of Strandberg[1] concludes that "In aerial photographs, oxygen-deficient water frequently appears black, or at least darker in tone and this appearance may be caused by the incomplete reduction of wastes by anaerobic bacteria." Thus his black-and-white photographs of waterbodies with low values of dissolved oxygen (DO) following a source of waste discharge are characterized by a plume discharge followed downstream by dark water tones.

Further values ascribed to photography in this manual include color photographs dealing with fish kills where the extent of damage can be better ascessed and the use of "false" color infrared photography where algal masses can be differentiated and, again, the low DO concentration is manifested by a color anomaly.

C Imagery Techniques

1 Utilization of the "mid" and "far" infrared wavelengths in the form of "imagery" has been found useful in analyzing for thermal pollution and its concomitant circulation and diffusion patterns for which "thermographs" can be described for large water bodies.[2] It is also possible to establish naturally occurring patterns of seasonal and diurnal thermal ranges for surface waters.

2 It has been ascertained, in principle, that it is possible to identify pollutants by study of spectral emissivity characteristics for particular wavelengths and this can be of value, for instance, when one is searching for a particular pollutant and the emissivity characteristics for the waterbody can be compared with known patterns for the specific pollutant.[2]

A hypothetical situation is plotted in Figure 8 where spectrometric values of polluted water discharge are compared to a "normal" pattern of the water body.

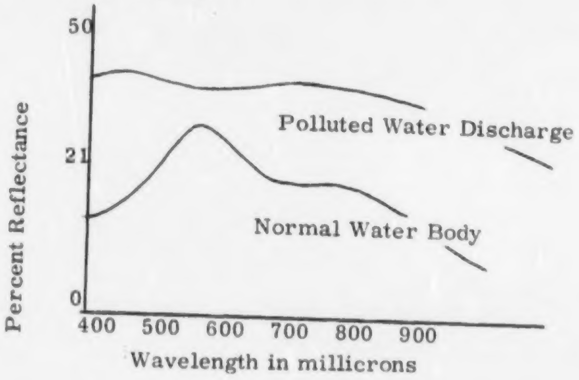

Figure 8

3 Utilization of radar and orbiting satellite platforms can produce imagery of great usefulness. The radar technique can precisely delineate shoreline characteristics without vegetative interferences and thus thoroughly image drainage basins. Satellite imagery and photography can obtain the same information but on a much broader scale and with great rapidity. Recent lines of thought have postulated that this orbiting scanner can be fitted with computing devices to render decisions upon analysis of its constant data collection.[3] Such decisions can be of immeasurable aid to the water conservationist who may require long range predictions based upon analysis of whole river basins.

IV SUMMARY

Development of aerial reconnaissance in pollution surveillance has proceeded in the areas of technique and interpretation. With regard to the techniques available most of the earlier problems have been overcome to a degree where available material is amenable to analysis. Such early problems as pitch and yaw of the aircraft, cloud cover, imagery instrumentation, etc., has been overcome to a large degree by available engineering skills and the need for improvement due to military needs. In the area of interpretation, however, much has yet to be accomplished to develop this tool as an extension of laboratory analysis which it will augment rather than replace. It is highly possible that remote sensing will take the final evolutionary form of fully automatic computerized operations. A variety of remote sensing devices can be envisioned to collectively contribute to a final printout of management decisions.

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3 Colwell, R.N. "Remote Sensing of Natural Resources." Scientific American. January 1968.

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Descriptors: Aerial Photography, Cameras, Mapping, Photogrammetry, Pollutants, Radar, Remote Sensing, Water Pollution

CASE PREPARATION AND COURTROOM PROCEDURE

I TYPES OF PROCEEDINGS IN WHICH WATER QUALITY EVIDENCE MAY BE USED

A Administrative Proceedings

1 Rule making

a Setting up of regulations having general application, e.g., stream classifications and implementation plan target dates

b Factors of safety and absolute prohibitions may be appropriate

2 Adjudications

a Determinations by agency having expertise with respect to particular discharge or discharger, e.g., approval of plans and specs and time schedule of a particular discharger

B Court Actions

1 Civil in behalf of state or federal government

a Actions to compel action or suspension of action - nuisance, health hazard, etc., --including court action following federal conference --hearing procedure

b Violations of Water Quality Standards

c Violations of Effluent Standards or discharge permits

d Tort or contract actions relating to design and/or operation of treatment facilities

2 Criminal (dependent on content of applicable statutes).

a Discharge of specific materials

b Discharges from specific industries

c Littering

d Discharges harmful to fish and/or crustaceans

e Discharges harmful to specific types of receiving waters

f Discharges of poisons

NOTE--In some of these situations doing the act may constitute the violation; in others proof of intent or knowledge of effects may also have to be proved.

3 Private actions for damages or to compel action

a Alleged harm to plaintiff, e.g., pollution of stream killing animals

C Procedural Matters

1 See Attached sheet "Administrative and Court Proceedings" on Burden of proof, fact finding, and methods of presentation of evidence.

D Classes of Evidence - General Rules

1 Facts - direct

a The material was floating from the outfall.

2 Derived values - expert testimony - test results and/or opinion as to effects

a The D.O. was zero; the waterway was polluted; the plant can be built in 6 months.

3 Hearsay

a Joe told me

- 4 Relevancy
- 5 Admissibility vs. weight
  - a Even if admissible, the weight to be given is up to fact finder--credibility.

E Admissibility of Results of Sampling and Testing (Numbers)

- 1 Sampling
  - a Chain of custody
  - b Tags, etc.
  - c Containers
  - d Place and time
  - e Retention of samples (Proving that the sample represents what is at issue in the action (relevancy), that there has been no opportunity for tampering; and availability of portions for analysis by other side (non-transitory criteria)).
- 2 Analysis
  - a Who performed (Can identity of each participant be shown?)
  - b Admission through supervisor - custodian
  - c Scientific acceptance of method. Is there a particular method required to be used by the agency?
  - d Propriety of conduct
  - e Retention of bench cards and other indicia of results. (Your attorney can make arrangements to substitute copies for originals).
- 3 Tests
  - a Comparison with actual conditions
  - b Mathematical models - how can a computer be cross-examined?

F Admissibility of Expert Opinion on Causes and Effects

- 1 Who has special knowledge - and of what particular areas?
- 2 Indicators
- 3 Significance of numerical determinations or observations
- 4 Consistency with own prior publications and testimony
- 5 Have underlying facts been or need to be proved--first hand information of this and/or comparable situations.
- 6 Use of treatises

G Conduct on the Witness Stand

- 1 General
  - a On direct - know what counsel will ask and let him know generally what you will answer, but don't make it sound rehearsed.
  - b Use layman's language to extent possible.
  - c Listen to question and answer it to best of your ability.
  - d Speak so that court reporter, judge, jury, and counsel can hear you.
  - e Speak in language that will be understood; don't talk down.
  - f Answer only what you are asked --don't volunteer; however, answer with precision.
  - g There is nothing wrong with asking to have a question repeated or rephrased.
  - h There is nothing wrong with saying that you consulted with your attorney before you testified, but beware of the question "Did Mr. X tell you what to say?"

i There is nothing wrong with thinking out your answer before responding.

j You are not expected to know all the answers--if you do not know, admit it.

k Don't attempt to answer questions outside your area of personal knowledge (hearsay) or beyond your expertise. (Your may be an expert on conducting laboratory tests, but not on epidimeological inferences from results).

l Don't try to answer before the judge rules on objection.

m Show that you are an impartial dispenser of information and/or opinion, not a protagonist.

n Don't be afraid to admit what may appear to be damaging.

2 If you are testifying as an expert:

a Establish qualifications -- give information relevant to your area of expertise -- educational (including this course?), work, publications, number of times you have testified previously.

b Differentiate between physical facts (measurements and observations) and opinion (derived values).

c Be prepared to discuss theory (including assumptions) instruments used, techniques(including choice of a particular technique), physical limitations and errors, interferences.

d If experiments were conducted, be able to justify both as to theory and relevancy to this litigation.

e If you're being paid to testify, admit it.

3 Scientific personnel as advisers to counsel:

a Review and refamiliarize self with materials before you discuss with your attorney.

b Be in a position to present all facts known to you simply and concisely: Who, What, When, Where, and Why, How.

c Don't overlook facts and/or test results because you don't think they're important. Let attorney decide what he needs.

d Use of standard report forms

e Ability to recommend additional witnesses with needed specialized knowledge

f Ability to aid in cross-examination of other side's experts and reconcile opinions and/or results

g Be candid - sometimes better not to start a lawsuit or accept a settlement than lose in the end.

H Non-Verbal Presentation of Evidence

1 Exhibits - including photographs

2 Summaries

3 Business and/or government records

a Prepared contemporaneously and in usual course of activities

4 Pre-prepared direct examination

a Usually limited to actions before ICC, FPC, and other federal agencies.

I Criminal Procedure

1 Privilege Against Self Incrimination (available only to persons)

a Warning and suspects

b Effect of duty to report spills

c Effect of duty to obtain license or permit and/or furnish operating reports

d Immunity from prosecution

2 Double Jeopardy

3 Unreasonable search and seizure

a Available to persons and corporations

4 Procedures and need for arrest and search warrants --possible cause

This outline was prepared by David I. Shedroff, Enforcement Analyst, Office of Enforcement and General Counsel, Cincinnati Field Investigations Center, 5555 Ridge Avenue, Cincinnati, OH 45268.

Descriptors: Courtroom Procedure, Law Enforcement, Legal Aspects, Sampling, Water Analysis, Water Pollution Control, Water Quality Standards

Administrative & Court Proceedings, and Excerpts from Revised Draft of Proposed Rules of Evidence for the United States Courts can be found on the following pages.

ADMINISTRATIVE & COURT PROCEEDINGS

Court or Agency	Fact Finder	Burden of Proof	Comments
State Pollution Control Agency	Agency	As per statute - usually weight of evidence.	Hearing may be conducted by hearing examiner, agency member, or full agency. Appeal may be on facts and law or law alone, depending on statute.
Rule making-adjudication			
Federal Water Pollution Control Act			
Conference	Head of agency		Reports acceptable.
Hearing	Hearing Board		Specific testimony.
Court	Judge		Uses prior material, and may take additional testimony.
Court			
Civil Case -- - for money only	Judge or jury	Weight of evidence	
- injunction			
preliminary or temporary	Judge	Must show immediate harm or danger.	Must also show likelihood of success at final hearing - bond required for non-government plaintiff.
permanent	Judge	Usually clear and convincing.	"Balance Equities"
- administrative appeal	Judge - whether "arbitrary and capricious" or substantial evidence.		Sometimes have complete new trial.
Criminal case includes penalties	Jury unless waived.	Beyond reasonable doubt.	Proof of intent may be required.

Excerpts from Revised Draft of Proposed
RULES OF EVIDENCE FOR THE UNITED STATES COURTS

GENERAL PROCEDURES

Rule 102.
PURPOSE AND CONSTRUCTION

These rules shall be construed to secure fairness in administration, elimination of unjustifiable expense and delay, and promotion of growth and development of the law of evidence to the end that the truth may be ascertained and proceedings justly determined.

Rule 101.
PRELIMINARY QUESTIONS

(a) Questions of Admissibility Generally. Preliminary questions concerning the qualification of a person to be a witness, the existence of a privilege, or the admissibility of evidence shall be determined by the judge, subject to the provisions of subdivision (b). In making his determination he is not bound by the rules of evidence except those with respect to privileges.

(b) Relevancy Conditioned on Fact. When the relevancy of evidence depends upon the fulfillment of a condition of fact, the judge shall admit it upon, or subject to, the introduction of evidence sufficient to support a finding of the fulfillment of the condition.

Rule 615.
EXCLUSION OF WITNESSES

At the request of a party the judge shall order witnesses excluded so that they cannot hear the testimony of other witnesses, and he may make the order of his own motion. This rule does not authorize exclusion of (1) a party who is a natural person, or (2) an officer or employee of a party which is not a natural person designated as its representative by its attorney, or (3) a person whose presence is shown by a party to be essential to the presentation of his cause.

Rule 611.
MODE AND ORDER OF INTERROGATION AND PRESENTATION

(a) Control by Judge. The judge may exercise reasonable control over the mode and order of interrogating witnesses and presenting evidence so as to (1) make the interrogation and presentation effective for the ascertainment of the truth, (2) avoid needless consumption of time, and (3) protect witnesses from harassment or undue embarrassment.

(b) Scope of Cross-Examination. A witness may be cross-examined on any matter relevant to any issue in the case, including credibility. In the interests of justice, the judge may limit cross-examination with respect to matters not testified to on direct examination.

Rule 613.

PRIOR STATEMENTS OF WITNESSES

(a) Examining Witness Concerning Prior Statement. In examining a witness concerning a prior statement made by him, whether written or not, the statement need not be shown or its contents disclosed to him at that time, but on request the same shall be shown or disclosed to opposing counsel.

JUDICIAL NOTICE

Rule 201.

JUDICIAL NOTICE OF ADJUDICATIVE FACTS

(b) Kinds of Facts. A judicially noticed fact must be one not subject to reasonable dispute in that it is either (1) generally known within the territorial jurisdiction of the trial court or (2) capable of accurate and ready determination by resort to sources whose accuracy cannot reasonably be questioned.

(g) Instructing Jury. The judge shall instruct the jury to accept as established any facts judicially noticed.

RELEVANCE

Rule 401.

DEFINITION OF "RELEVANT EVIDENCE"

"Relevant evidence" means evidence having any tendency to make the existence of any fact that is of consequence to the determination of the action more probable or less probable than it would be without the evidence.

Rule 402.

RELEVANT EVIDENCE GENERALLY ADMISSIBLE; IRRELEVANT EVIDENCE INADMISSIBLE

All relevant evidence is admissible, except as otherwise provided by these rules, by other rules adopted by the Supreme Court, by Act of Congress, or by the Constitution of the United States. Evidence which is not relevant is not admissible.

COMPETENCY OF WITNESSES

Rule 601.

GENERAL RULE OF COMPETENCY

Every person is competent to be a witness except as otherwise provided in these rules.

Rule 602.

LACK OF PERSONAL KNOWLEDGE

A witness may not testify to a matter unless evidence is introduced sufficient to support a finding that he has personal knowledge of the matter. Evidence to prove personal knowledge may, but need not, consist of the testimony of the witness himself. This rule is subject to the provisions of Rule 703, relating to opinion testimony by expert witnesses.

EXPERT TESTIMONY

Rule 702.

TESTIMONY BY EXPERTS

If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise.

Rule 703.

BASES OF OPINION TESTIMONY BY EXPERTS

The facts or data in the particular case upon which an expert bases an opinion or inference may be those perceived by or made known to him at or before the hearing. If of a type reasonably relied upon by experts in the particular field in forming opinions or inferences upon the subject, the facts or data need not be admissible in evidence.

Rule 705.

DISCLOSURE OF FACTS OR DATA UNDERLYING EXPERT OPINION

The expert may testify in terms of opinion or inference and give his reasons therefore without prior disclosure of the underlying facts or data, unless the judge requires otherwise. The expert may in any event be required to disclose the underlying facts or data on cross-examination.

Rule 706.

COURT APPOINTED EXPERTS

(a) Appointment. The judge may on his own motion or on the motion of any party enter an order to show cause why expert witnesses should not be appointed, and may request the parties to submit nominations. The judge may appoint any expert witnesses agreed upon by the parties, and may appoint witnesses of his own selection. An expert witness shall not be appointed by the judge unless he consents to act. A witness so appointed shall be informed of his duties by the judge in writing, a copy of which shall be filed with the clerk, or at a conference in which the parties shall have opportunity to participate. A witness so appointed shall advise the parties of his findings, if any; his deposition may be taken by any party; and he may be called to testify by the judge or any party. He shall be subject to cross-examination by each party, including a party calling him as a witness.

HEARSAY

Rule 801.

DEFINITIONS

The following definitions apply under this Article:

(a) Statement. A "statement" is (1) an oral or written assertion or (2) nonverbal conduct of a person, if it is intended by him as an assertion.

(b) Declarant. A "declarant" is a person who makes a statement.

(c) Hearsay. "Hearsay" is a statement, other than one made by the declarant while testifying at the trial or hearing, offered in evidence to prove the truth of the matter asserted.

Rule 802.

HEARSAY RULE

Hearsay is not admissible except as provided by these rules or by other rules adopted by the Supreme Court or by Act of Congress.

Rule 803.

HEARSAY EXCEPTIONS: AVAILABILITY OF DECLARANT IMMATERIAL

The following are not excluded by the hearsay rule, even though the declarant is available as a witness:

(5) Recorded Recollection. A memorandum or record concerning a matter about which a witness once had knowledge but now has insufficient recollection to enable him to testify fully and accurately, shown to have been made when the matter was fresh in his memory and to reflect that knowledge correctly. If admitted, the memorandum or record may be read into evidence but may not itself be received as an exhibit unless offered by an adverse party.

(6) Records of Regularly Conducted Activity. A memorandum, report, record, or data compilation, in any form, of acts, events, conditions, opinions, or diagnoses, made at or near the time by, or from information transmitted by, a person with knowledge, all in the course of a regularly conducted activity, as shown by the testimony of the custodian or other qualified witness, unless the sources of information or other circumstances indicate lack of trustworthiness.

(18) Learned Treatises. To the extent called to the attention of an expert witness upon cross-examination or relied upon by him in direct examination, statements contained in published treatises, periodicals, or pamphlets on a subject of history, medicine, or other science or art, established as a reliable authority by the testimony or admission of the witness or by other expert testimony or by judicial notice. If admitted, the statements may be read into evidence but may not be received as exhibits.

IDENTIFICATION OF PERSONS AND SAMPLES

Rule 901.

REQUIREMENT OF AUTHENTICATION OR IDENTIFICATION

(a) General Provision. The requirement of authentication or identification as a condition precedent to admissibility is satisfied by evidence sufficient to support a finding that the matter in question is what its proponent claims.

(b) Illustrations. By way of illustration only, and not by way of limitation, the following are examples of authentication or identification conforming with the requirements of this rule:

(1) Testimony of Witness with Knowledge. Testimony that a matter is what it is claimed to be.

(3) Comparison by Trier or Expert Witness. Comparison by the trier of fact or by expert witnesses with specimens which have been authenticated.

(9) Process or System. Evidence describing a process or system used to produce a result and showing that the process or system produces accurate result.

ADMISSIBILITY AND PROOF OF SPECIAL MATTERS

Rule 406.

HABIT; ROUTINE PRACTICE

(a) Admissibility. Evidence of the habit of a person or of the routine practice of an organization, whether corroborated or not and regardless of the presence of eyewitnesses, is relevant to prove that the conduct of the person or organization on a particular occasion was in conformity with the habit or routine practice.

(b) Method of Proof. Habit or routine practice may be proved by testimony in the form of an opinion or by specific instances of conduct sufficient in number to warrant a finding that the habit existed or that the practice was routine.

Rule 612

WRITING USED TO REFRESH MEMORY

If a witness uses a writing to refresh his memory, either before or while testifying, an adverse party is entitled to have it produced at the hearing, to inspect it, to cross-examine the witness thereon, and to introduce in evidence those portions which relate to the testimony of the witness.

Rule 1006.

SUMMARIES

The contents of voluminous writings, recordings, or photographs which cannot conveniently be examined in court may be presented in the form of a chart, summary, or calculation. The originals, or duplicates, shall be made available for examination or copying, or both, by other parties at a reasonable time and place. The judge may order that they be produced in court.